Mogućnosti rane dijagnostike kronične opstruktivne plućne bolesti u populaciji pod rizikom

Vrbica, Žarko

SVEUČILIŠTE U SPLITU

MEDICINSKI FAKULTET

Žarko Vrbica

MOGUĆNOSTI RANE DIJAGNOSTIKE KRONIČNE OPSTRUKTIVNE PLUĆNE BOLESTI U POPULACIJI POD RIZIKOM

Doktorska disertacija

Split, 2024.

Doktorska disertacija sadrži rezultate znanstvenih istraživanja provedenih u sklopu projekta MARKO (MArkeri Rane dijagnostike bolesti u ispitanika rizičnih za razvoj KOPB-a)

VODITELJI ISTRAŽIVANJA: prof. dr. sc. Davor Plavec, prim.mr.sc. Žarko Vrbica

ZAHVALJUJEM

Roditeljima koji su me usmjeravali i uputili u život

Obitelji koja me je podržavala i poticala

Kolegama i suradnicima u projektu na entuzijazmu i zalaganju

SADRŽAJ

1. POPIS OZNAKA I KRATICA

MARKO: MArkeri Ranog otkrivanja KOPB-a

KOPB: Kronična opstruktivna plućna bolest

PKY: Broj godina pušenja (cigareta dnevno x godine pušačkog staža/20)

GOLD (Global Initiative for Chronic Obstructive Lung Disease): Globalna inicijativa za KOPB

SGRQ (St. George's Respiratory Questionnaire): St. George respiratorni upitnik za procjenu kvalitete života

CAT (COPD Assessment Test): Upitnik procjene kontrole KOPB-a

FVC: Forsirani vitalni kapacitet

FEV_1: Forsirani izdahnuti volumen u prvoj sekundi

FEV_6: Forsirani izdahnuti volumen tijekom prvih 6 sekundi

EBT (Exhaled Breath Temperature): Temperatura izdahnutog zraka

ERS (European Respiratory Society): Europsko respiratorno društvo

ATS (American Thoracic Society): Američko torakalno društvo

mMRC (Modified Medical Research Council Dyspnea Scale): Modificirana ljestvica zaduhe Vijeća za medicinska istraživanja

AUC (Area Under the Curve): Površina pod krivuljom

LLN (Lower Limit of Normal): Donja granica referentnih vrijednosti

CI (Confidence Interval): Interval pouzdanosti

PRISm (Preserved Ratio Impaired Spirometry): Spirometrijski poremećaj s očuvanim omjerom

2. PREGLED OBJEDINJENIH RADOVA

Doktorska disertacija nastala je objedinjenjem pet znanstvenih članaka:

1. Vrbica Ž, Labor M, Gudelj I, Labor S, Jurić I, Plavec D; MARKO study group. Early detection of COPD patients in GOLD 0 population: an observational non-interventional cohort study - MARKO study. BMC Pulm Med. 2017 Feb 10;17(1):36. doi:10.1186/s12890-017-0378-6
2. Vrbica Z, Labor M, Koscec Duknic A, Radosevic-Vidacek B, Gudelj I, Labor S, Juric I, Calverley PM, Plavec D. Development and the initial validation of a new self-administered questionnaire for an early detection of health status changes in smokers at risk for chronic obstructive pulmonary disease (MARKO questionnaire). Croat Med J. 2016 Oct 31;57(5):425-433. doi:10.3325/cmj.2016.57.425
3. Labor M, Vrbica Z, Gudelj I, Labor S, Plavec D. Diagnostic accuracy of a pocket screening spirometer in diagnosing chronic obstructive pulmonary disease in general practice: a cross sectional validation study using tertiary care as a reference. BMC Fam Pract. 2016 Aug 19;17(1):112. doi:10.1186/s12875-016-0518-8
4. Labor M, Vrbica Ž, Gudelj I, Labor S, Jurić I, Plavec D. Exhaled Breath Temperature as a Novel Marker of Future Development of COPD: Results of a Follow-Up Study in Smokers. COPD. 2016 Dec;13(6):741-749. doi:10.3109/15412555.2016.1164129
5. Vrbica Z, Steiner J, Labor M, Gudelj I, Plavec D. Breathlessness and “exacerbation” questions predictive for incident COPD (MARKO study): data after two years of follow-up. PeerJ. 2023 Dec; DOI 10.7717/peerj.16650

2.1. UVOD

Kronična opstruktivna plućna bolest (KOPB) jedan je od glavnih uzroka pobolijevanja i smrtnosti u svijetu i zahvaća oko jedne desetine odrasle (ili 1/20 svjetske) populacije, oko 400 milijuna ljudi (1). Kao uzrok smrti nalazi se na trećem mjestu uz tendenciju daljeg porasta u slijedećem desetljeću (2). Svjetska zdravstvena organizacija definira problem rane dijagnostike i liječenja KOPB-a kao jednu od značajnih otvorenih potreba zdravstvenog sustava (3).

Mortalitet od KOPB-a je nizak u mlađoj životnoj dobi do 45. godina starosti, ali naglo raste nakon 65-te godine (4). Težina bolesti, akutna pogoršanja (egzacerbacije) te učestali komorbiditeti imaju značajan ekonomski i javno-zdravstveni utjecaj. Najznačajniji čimbenik rizika za nastanak KOPB-a je pušenje, ali samo oko 1/3 pušača razvije KOPB tijekom života. Zbog svojeg progresivnog tijeka, KOPB dovodi do rane pojave invalidnosti i skraćenja životnog vijeka. Zaustavljanje ili usporavanje pogoršanja KOPB-a je još uvijek neispunjena potreba zdravstvenog sustava (2).

KOPB je heterogena bolest koja se sastoji od kombinacije različitih patofizioloških procesa čiji udio se razlikuje od bolesnika do bolesnika. Među njima su najznačajniji: ometeni razvoj pluća u djetinjstvu i mladosti (5,6), kumulativno oštećenje pluća izazvano duhanskim dimom te zagađenjem zraka (u kući, na radnom mjestu i u okolišu), respiratorne infekcije te remodeliranje pluća (7).

Iako postoje mjere liječenja koje mogu usporiti progresiju KOPB-a, od kojih je najznačajnija prestanak pušenja, studije pokazuju da su one značajno učinkovitije ukoliko se počnu provoditi u ranoj fazi bolesti (8,9,10,11,12). Rano postavljanje dijagnoze i/ili otkrivanje „rizičnih“ pušača (one 1/3 koja će razviti KOPB) bi moglo omogućiti raniju intervenciju i dovesti do bitno boljih ishoda liječenja u vidu usporenja pogoršanja plućne funkcije, poboljšanja kvalitete života, podnošenja napora i smanjenja težine i učestalosti egzacerbacija.

Problem ranog otkrivanja KOPB-a je u tome da pušači često svoje tegobe (kašalj/iskašljaj/zaduha) povezuju s pušenjem, a ne sa samom bolesti i kasno se zbog tih tegoba javljaju liječniku te se dijagnoza postavlja u uznapredovanoj fazi KOPB-a. Osim navedenog, početkom bolesti plućna funkcija mjerena spirometrijom često nije poremećena jer je referentni interval razmjerno širok. Time se dijagnoza postavlja kad već postoji klinički značajno ireverzibilno oštećenje plućne funkcije. Kako bi se rezultati liječenja poboljšali, bilo bi potrebno uspostaviti probir u rizičnoj populaciji koji bi doveo do ranijeg otkrivanja KOPB-a, a samim time i do ranijeg započinjanja liječenja (13,14,15).

Trenutno je jedini dijagnostički postupak koji je primjeren ranom otkrivanju KOPB-a spirometrija koja je ujedno i zlatni standard pri postavljanju dijagnoze (16,17). Aktualne smjernice za dijagnostiku i liječenje KOPB-a ne preporučuju masovni probir opće populacije već zagovaraju rano otkrivanje oboljelih u rizičnoj skupini (rano pronalaženje oboljelih). Važno je naglasiti da lošija spirometrija bez znakova bolesti ne predstavlja KOPB. Zbog toga se trenutno bolest kod većine bolesnika dijagnosticira u kasnijoj fazi bolesti kada se bolesnik javlja liječniku zbog uznapredovanih tegoba. Kako se radi o kroničnoj progresivnoj bolesti, u toj fazi je cilj liječenja usporiti pogoršanje bolesti i ne može se više postići oporavak izgubljene plućne funkcije. Zbog navedenog se sve više inzistira na terminima Pre-KOPB-a (simptomatske osobe bez detektabilnih strukturalnih promjena pluća ili opstrukcije dišnih putova) i PRISm (spirometrijski poremećaj s očuvanim omjerom) kod kojih je došlo do sniženja FEV1 (forsirani izdahnuti volumen u prvoj sekundi), ali bez redukcije omjera FEV1/FVC (forsirani vitalni kapacitet) ispod 70%. Ti se pojmovi uvode zbog naglaska na značaju ranih promjena u razvoju KOPB-a i insistira se na provođenju prospektivnih studija na tim populacijama (2).

Mali dišni putovi su čini se ishodište bolesti i zahvaćeni su u samom početku bolesti, ali dijagnosticiranje njihovog oštećenja nije moguće standardnom spirometrijom zbog njezine niske osjetljivosti za mjerenje navedenih promjena. Zbog toga nedostaju podaci o tijeku bolesti kod ranih oštećenja kao i o mogućim terapijskim postupcima njihovog zbrinjavanja. Također nema prospektivnih ispitivanja razvoja oštećenja malih dišnih putova, emfizema i bronhitičnih promjena tijekom vremena kao niti utjecaja terapijskih postupaka na njihovu dinamiku jer je potrebno obuhvatiti veliki broj rizičnih pojedinaca te koristiti za praćenje kompleksne te često i invazivne i skupe metode (kompjutorizirana tomografija pluća visoke rezolucije).

Projekt MARKO je definiran na temelju navedenih postavki i predstavlja prospektivno istraživanje s ciljem identificiranja osoba iz skupine rizičnih pušača kod kojih će se tijekom vremena razviti KOPB.

Respiracijski simptomi javljaju se kod preko 50% pušača kod kojih se spirometrijski još ne može postaviti dijagnoza KOPB-a. Stupanj tih tegoba se može kvantificirati odgovarajućim strukturiranim upitnicima. U našem ispitivanju smo koristili novo konstruirani upitnik temeljen na kvaliteti života povezanoj sa zdravljem (HRQoL, od engl. *health related quality of life*).(18) Praćenje bolesnika s pozitivnim odgovorom na pitanja iz upitnika koja bi se pokazala povezanima s progresijom pogoršanja plućne funkcije i time definirati bolesnike kod kojih bi se ranom intervencijom moglo prevenirati ili odgoditi razvoj KOPB-a.

Postavlja se pitanje mogućnosti otkrivanja početnih patofizioloških procesa u podlozi oštećenja pluća prije nego nastane nepovratno oštećenje pluća (19,20,21,22). Neinvazivno mjerenje temperature izdaha pokazalo je potencijal u mjerenju podležećeg upalnog procesa u različitim respiratornim bolestima (astma, KOPB, karcinomi bronha). Kako se u podlozi KOPB-a nalazi kronična upalna reakcija na udisanje štetnih čimbenika kao što je duhanski dim, mjerenje temperature izdahnutog zraka i razlike nakon udisanja duhanskog dima moglo bi ukazati na one pušače kod kojih postoji aktivna upalna reakcija i predvidjeti nastanak oštećenja pluća ukoliko nastave s pušenjem (23).

Novija istraživanja upućuju na genetsku podlogu razlike u osjetljivosti pojedinaca na štetne učinke pušenja, ali ti rezultati nisu potvrđeni prospektivnim istraživanjima (20). U našem projektu su uzeti uzorci biološkog materijala (krvi) i pohranjeni za naknadnu analizu prema kasnije definiranim kohortama prema nastanku oštećenja plućne funkcije i razvoju KOPB-a.

2.1.1. CILJEVI ISTRAŽIVANJA

Pojava tegoba i smanjenja kvalitete života pušača često prethodi nastanku KOPB-a. Navedeno bi se moglo iskoristiti za rano otkrivanje bolesnika prije nego dođe do nepovratnog oštećenja plućne funkcije, ali ne postoje podaci koji bi definirali kako provesti navedeno. Kako je broj rizičnih osoba za razvoj bolesti izuzetno velik (>1,5 milijardi), a samo 1/3 razvije KOPB tijekom svog životnog vijeka nije moguće koristiti kompleksne i skupe metode ranog otkrivanja. Stoga su u svakodnevnoj praksi potrebni jednostavni alati pomoću kojih bi mogli rano postaviti sumnju na početak KOPB-a kod pojedinog pušača i usmjeriti tako otkrivene bolesnike u rani dijagnostički postupak i liječenje. Na temelju navedenih pretpostavki, mogućnosti ranog otkrivanja rizičnih pušača i značajno boljeg uspjeha ranog liječenja u navedenoj skupini, odlučili smo razviti novi alat koji bi se mogao koristiti sam ili u sklopu drugih bioloških biljega i testirati ga u populaciji rizičnih pušača ili bivših pušača kod kojih postoji značajna izloženost štetnim učincima pušenja (u našem istraživanju definiranih kao izloženost od >20 pky i dobi od 40-65 godina), a ne zadovoljavaju kriterije za postavljanje dijagnoze KOPB-a. Ovaj alat bi trebao biti primjenljiv na globalnoj razini, jeftin, jednostavan, lako provediv, s umjerenim do visokim stupnjem osjetljivosti i visokom specifičnosti. Kako su dostupni upitnici za procjenu kvalitete života oboljelih od KOPB-a komplicirani i nisu razvijeni u cilju ranog otkrivanja bolesti, mi smo na temelju vlastitih iskustava i podataka iz literature razvili jednostavan upitnik usmjeren prema ranom otkrivanju promjena povezanih s KOPB-om. U cilju definiranja, razvoja i provjere navedenog upitnika pokrenut je projekt MARKO (MArkeri Rane dijagnostike bolesti u ispitanika rizičnih za razvoj KOPB-a). Primarni cilj istraživanja je razvoj novodizajniranog MARKO upitnika i procjena njegove upotrebljivosti u ranom otkrivanju pojedinaca kod kojih postoji povećan rizik nastanka KOPB-a. Sekundarni ciljevi istraživanja su evaluacija psihometrijskih karakteristika MARKO upitnika, usporedna učinkovitost u ranoj dijagnostici u kombinaciji s COPD-6 mjeračem plućne funkcije, procjena brzine pogoršanja plućne funkcije kod bolesnika s elementima KOPB-a, ali bez spirometrijske potvrde bolesti (prijašnji GOLD „0“), odrediti prevalenciju komorbiditeta u rizičnoj populaciji, određivanje dijagnostičkih parametara koji najbolje predviđaju rano pogoršanje plućne funkcije, određivanje prevalencije ranih faza bolesti (GOLD „0“ i „1“) u rizičnoj populaciji.

Primarni cilj istraživanja je razvoj i validacija MARKO upitnika koji bi se sam ili u kombinaciji s drugim biljezima aktivnosti bolesti mogao koristiti u identificiranju rizičnih pušača kod kojih će doći do razvoja KOPB-a.

Sekundarni ciljevi istraživanja su:

a) Evaluacija psihometrijskih karakteristika MARKO upitnika.-a

b) Mogućnost diskriminacije različitih skupina bolesnika (asimptomatski pušači, simptomatski pušači, KOPB I i II stupnja prema globalnoj inicijativi za KOPB - GOLD) putem MARKO upitnika samog ili u kombinaciji s mjerenjem plućne funkcije putem COPD-6 spirometra.

c) Odrediti stupanj pogoršanja plućne funkcije kod simptomatskih pušača tijekom praćenja.

d) Odrediti prevalenciju pridruženih bolesti u ispitivanoj populaciji.

e) Identifikacija parametara koji najbolje otkrivaju rane poremećaje u KOPB-u

f) Usporedba MARKO upitnika s postojećim dijagnostičkim postupcima evaluacije KOPB-a (anamnestički podaci, fizikalni nalaz, mjerenje plućne funkcije, šestominutni test hoda, St. George's Respiratory Questionnaire – SGRQ i COPD Assessment Test – CAT)

g) Procjena prevalencije pojedinih stupnjeva KOPB-a kod pušača bez do sada dijagnosticiranog-a KOPB-a.

2.2. MATERIJALI I METODE

MARKO upitnik je novo-razvijeni upitnik od strane grupe eksperata koju su činila tri pulmologa s iskustvom u liječenju KOPB-a i dva psihologa. Sastoji se od 18 pitanja koja definiraju tegobe i učestalost smetnji u definiranom vremenskom razdoblju. Ukupni rezultat upitnika može biti 0-57 bodova pri čemu veći broj upućuje na izraženije tegobe. Navedeni upitnik je korišten usporedno s postojećim upitnicima za procjenu kvalitete života oboljelih od KOPB-a, mjerenjem plućne funkcije, temperature izdahnutog zraka, biljega upalnog procesa i arhiviranjem uzoraka za molekularnu analizu. MARKO ispitivanje je planirano u dvije faze, a u ovim objedinjenim radovima su prikazani rezultati obje faze ispitivanja. U prvu fazu je uključeno 450 ispitanika koji su probrani od strane 25 nadležnih liječnika obiteljske medicine i 7 tercijarnih zdravstvenih ustanova.

Ispitanici su regrutirani od strane nadležnih liječnika obiteljske medicine među bolesnicima koji su posjetili svog liječnika zbog problema nepovezanog s respiracijskim tegobama. Ukoliko su se nalazili u rizičnoj skupini, ispunili bi informirani pristanak prije uključivanja u ispitivanje. Kriteriji uključivanja su bili: pušač/bivši pušač s >20 pušačkih godina (pky, od engl. *pack-years*) u dobi od 40-65 godina i bez postavljene dijagnoze KOPB-a. Isključni kriteriji su bili: klinički značajna kronična bolest (kardiovaskularna, cerebrovaskularna, dijabetes, hepatitis, nefropatija, kronična dijaliza, sustavne bolesti ili zloćudne bolesti) koja značajno utječe na kvalitetu života, bolničko liječenje tijekom posljednja 3 mjeseca neovisno o uzroku, infarkt miokarda, moždani udar ili tranzitorna ishemijska ataka tijekom posljednjih 6 mjeseci, dijagnoza astme ili nemogućnost provođenja nekog od planiranih studijskih postupaka.

Po uključenju u istraživanje, bolesnici bi samostalno ispunili MARKO upitnik u ordinaciji liječnika obiteljske medicine i proveli mjerenje plućne funkcije s COPD6 spirometrom.

Dva do četiri tjedna nakon navedene inicijalne obrade, ispitanik je bio naručen u jedan od 7 tercijarnih centara na dalje ispitivanje tijekom kojega je učinjena pulmološka obrada uz uzimanje anamnestičkih podataka, fizikalni pregled i ponovno ispunjavanje MARKO upitnika. Dodatno bi ispunio SGRQ i CAT upitnik, izmjerena je temperatura izdahnutog zraka prije i nakon popušene cigarete i uzeta je krv koja je pohranjena za dalju obradu. U sklopu obrade je učinjeno mjerenje plućne funkcije (spirometrija s farmakodinamskim testom sa salbutamolom, difuzijski kapacitet za CO) i šestominutni test hoda. Nakon toga je bolesnik prema GOLD i ATS (American Thoracic Society) smjernicama kategoriziran u skupinu zdravih pušača ili oboljelih od KOPB-a s definiranjem stupnja bolesti.

U drugu fazu su uključeni ispitanici koji su u prvom stupnju procijenjeni kao „zdravi“ pušači, simptomatski pušači (prijašnji GOLD „0“) ili KOPB GOLD I stupnja i oni su praćeni i ponovno evaluirani nakon 2 godine (±2 mjeseca) od strane istog educiranog pulmologa. Postavljanje dijagnoze KOPB-a je provođeno u tercijarnoj zdravstvenoj ustanovi od strane istog pulmologa na temelju kliničkog pregleda i plućne funkcije sukladno GOLD preporukama. Incidencija novo-dijagnosticiranog KOPB-a nakon 2 godine praćenja je korištena za procjenu dijagnostičkih parametara koji su najosjetljiviji za otkrivanje ranih promjena u KOPB-u kao i u određivanju prediktabilnosti „MARKO“ upitnika samog ili u kombinaciji s drugim biljezima za razvoj ranih promjena u KOPB-u. Primarni cilj ovih analiza je bilo procjenjivanje prediktabilnosti novo-konstruiranog upitnika za samoprocjenu kvalitete života u KOPB-u (MARKO) i njegovih domena samih ili u kombinaciji s drugim biljezima (temperatura izdahnutog zraka, plućna funkcija, upalni biljezi) u identifikaciji ispitanika iz rizične skupine koji će razviti KOPB tijekom dvije godine praćenja. Sekundarni cilj je bio određivanje postotka progresije ispitanika iz GOLD „0“ stupnja u KOPB-u tijekom dvije godine.

Projekt MARKO je odobren od strane etičkog povjerenstva Dječje bolnice Srebrnjak (broj 04/2010 od 17.06.2010) i Kliničkog bolničkog centra Osijek (broj: 25-1:10359-3/2014 od 23.07.2014) i proveden prema pravilima dobre kliničke prakse sukladno Helsinškoj deklaraciji i relevantnim međunarodnim i lokalnim zakonskim propisima.

2.2.1. STATISTIČKA OBRADA

Analiza podataka rađena je uporabom STATISTICA ver. 12 (StatSoft Inc. OK, USA), MedCalc Statistical software ver. 16.8.4 (MedCalc Software bvba, Ostend, Belgium).

Analiza podataka fokusirana je na usporedbu pojedinih grupa ispitanika prema rezultatima MARKO upitnika, SGRQ, CAT i to hi-kvadrat testom, Fisherovim testom, Mann-Whitney U-testom i Kruskal-Wallis ANOVA testom. Rezultati MARKO upitnika su analizirani s Cronbach alfa, Linovim testom konkordance i Spearmanovim koeficijentom korelacije.

2.3. REZULTATI

2.3.1. SAŽETAK OBJEDINJENIH REZULTATA

U prvom radu je definiran problem i cilj istraživanja uz analizu trenutne problematike KOPB-a i stavljanje naglaska na otkrivanje ranih promjena u KOPB-u i otkrivanja rizičnih bolesnika u skupini „zdravih pušača“ što do tada nije bilo aktualno prema važećim smjernicama. Detekcija navedenog problema kasne dijagnostike KOPB-a i prijedloga za njegovo rješavanje je značajan doprinos shvaćanju problema KOPB-a i načinu pristupa njegovom rješenju što je vidljivo i iz niza kasnijih radova (24,25) koji upućuju na isti problem s različitim preporukama za njegovo rješavanje te je ovaj rad jedan od prvih koji su definirali taj problem kasnog otkrivanja patofizioloških procesa u nastanku KOPB-a u ranoj fazi. Također su predloženi i neki novi postupci koji bi mogli pomoći u otkrivanju rizičnih pušača, prvenstveno novo formirani MARKO upitnik i mjerenje temperature izdahnutog zraka.

U drugom radu je predstavljen MARKO upitnik i njegova primarna validacija u ispitivanoj skupini rizičnih bolesnika. MARKO upitnik je pokazao dobru unutarnju konzistenciju i primjenjivost u uvjetima ambulante obiteljske medicine kao i pouzdanost pri samostalnom ispunjavanju od strane ispitanika. Uz navedeno, pokazao je i značajnu korelaciju s prije validiranim upitnicima za procjenu KOPB-a (SGRQ, CAT) koji se upotrebljavaju u procjeni kontrole dijagnosticirane KOPB-om. SGRQ je složeni upitnik koji se primjenjuje u znanstvenim ispitivanjima KOPB-a, a njegova šira primjena je ograničena zahtjevnošću samog upitnika i potrebom za stručnom pomoći pri ispunjavanju. Postojanje jednostavnijeg upitnika (MARKO) kojega bi bolesnik mogao samostalno ispunjavati, a koji korelira s rezultatima SGRQ bi moglo značajno olakšati znanstvena ispitivanja KOPB-a kao i redovito praćenje bolesnika u svakodnevnoj praksi. Iako je kod svih upitnika (SGRQ, CAT i MARKO) nađena značajna varijanca između pojedinih skupina ispitanika („zdravi“ pušači, simptomatski pušači, bolesnici s KOPB GOLD I i II), jedino je kod upitnika MARKO nađena statistički značajna razlika u srednjem rezultatu u usporedbi s ostale tri skupine ispitanika što upućuje na mogućnost uporabe MARKO upitnika u otkrivanju rizičnih pušača u ranoj fazi KOPB-a prije mogućnosti postavljanja dijagnoze spirometrijom.

U trećem radu su prikazani rezultati analize ispitivane populacije prema plućnoj funkciji. Značajan doprinos je otkriće gotovo 20% bolesnika s KOPB-om u ispitivanoj rizičnoj populaciji, a koji nisu bili dijagnosticirani u rutinskom radu što indicira uvođenje aktivnijeg pristupa u otkrivanju tih bolesnika. Mjerenje plućne funkcije priručnim COPD6 spirometrom (4000 COPD-6™ Respiratory

Monitor, Vitalograph Ltd., Buckingham, UK) u ordinaciji liječnika obiteljske medicine pokazalo je umjerenu pouzdanost s visokom specifičnošću, ali niskom osjetljivosti za otkrivanje KOPB-a. Uređaj se može pouzdano koristiti u ordinaciji liječnika obiteljske medicine uz rezultate usporedive s onima postignutim u kliničkim uvjetima što čini razliku prema cjelovitoj spirometriji. Također, veliki dio bolesnika može se klasificirati prema tim rezultatima te se može smatrati kako omjer FEV1/FEV6 >0,85 s velikom sigurnosti može isključiti postojanje KOPB-a dok se omjer FEV1/FEV6 <0,7 može smatrati potvrdom dijagnoze KOPB-a te bi se samo rizični bolesnici s omjernom FEV1/FEV6 0,7-0,85 trebali dalje dijagnostički obrađivati što bi značajno pojednostavilo dijagnostiku KOPB-a i omogućilo otkrivanje oboljelih u rizičnoj skupini i ranije započinjanje liječenja.

U četvrtom radu je naglašen značaj mjerenja temperature izdahnutog zraka kao biljega aktivnosti upalnog procesa u KOPB-u. Nepostojanje pouzdanih biljega aktivnosti patofizioloških procesa u podlozi nastanka oštećenja pluća u KOPB-u je jedna od osnovnih prepreka ranom otkrivanju bolesnika koji će razviti teži oblik bolesti i kod kojih treba provesti ranu terapijsku intervenciju. U ovom radu smo dokazali povezanost s promjenom temperature izdahnutog zraka prije i nakon pušenja cigarete sa stupnjem aktivnosti KOPB-a. Također smo definirali promjenu temperature izdahnutog zraka kao jedan od prvih bio-markera osjetljivosti na štetne učinke duhanskog dima u rizičnoj populaciji.

U petom radu smo potvrdili značaj mjerenja temperature izdahnutog zraka u praćenju aktivnosti KOPB-a i verificirali moguću ulogu praćenja razlike temperature izdahnutog zraka prije i nakon inhalacije duhanskog dima u svrhu predviđanja rizika za razvoj KOPB-a u rizičnoj populaciji. Također smo otvorili novo područje istraživanja značaja neregistriranih/blagih pogoršanja KOPB-a kod bolesnika koji još ne zadovoljavaju spirometrijske kriterije za KOPB u razvoju i progresiji bolesti. Pokazali smo da rizični ispitanici koji imaju učestalu potrebu za liječenjem respiracijskih infekcija s antibioticima imaju veći rizik nastanka KOPB-a od onih koji nisu imali takve probleme.

2.3.2. Rezultati rada: „Early detection of COPD patients in GOLD 0 population: an observational non-interventional cohort study - MARKO study.“

U ovom radu je definiran problem i cilj istraživanja uz analizu trenutne problematike KOPB-a. KOPB je uspoređen s drugim kroničnim bolestima kod kojih je otkrivanje bolesti u najranijoj fazi i uvođenje terapijske intervencije u toj fazi dovelo do značajnog smanjivanja morbiditeta i mortaliteta. Tada smo (koncem 2010) postavili tezu kako definiranje KOPB-a tek nakon razvoja značajnog stupnja oštećenja plućne funkcije ne može bitno doprinijeti tom cilju već je potrebno iznaći nove načine što ranijeg otkrivanja bolesti već u samom početku patološkog procesa koji dovodi do oštećenja plućne funkcije i razvoja komorbiditeta. Otkrivanje rizičnih bolesnika u skupini „zdravih pušača“ tada nije bilo aktualno prema važećim smjernicama. Detekcija navedenog problema kasne dijagnostike KOPB-a i prijedloga za njegovo rješavanje značajan je doprinos shvaćanju problema KOPB-a i načinu pristupa njegovom rješenju što je vidljivo i iz niza kasnijih radova koji upućuju na isti problem s različitim preporukama za njegovo rješavanje. Ovaj rad je jedan od prvih koji su na ovaj način definirali taj problem.

Ideja uporabe upitnika o kvaliteti života u otkrivanju ranih poremećaja u KOPB-u je također etablirana ovim člankom. Uz navedenu ideju predložen je i novi jednostavniji upitnik u procjeni kvalitete života pušača (MARKO upitnik).

Ovdje je po prvi puta prezentirana ideja uporabe mjerenja temperature izdahnutog zraka u procjeni upalne aktivnosti u ranom KOPB-u kao i način njene uporabe u tu svrhu.

Protokol MARKO ispitivanja prikazan je na slici 1.

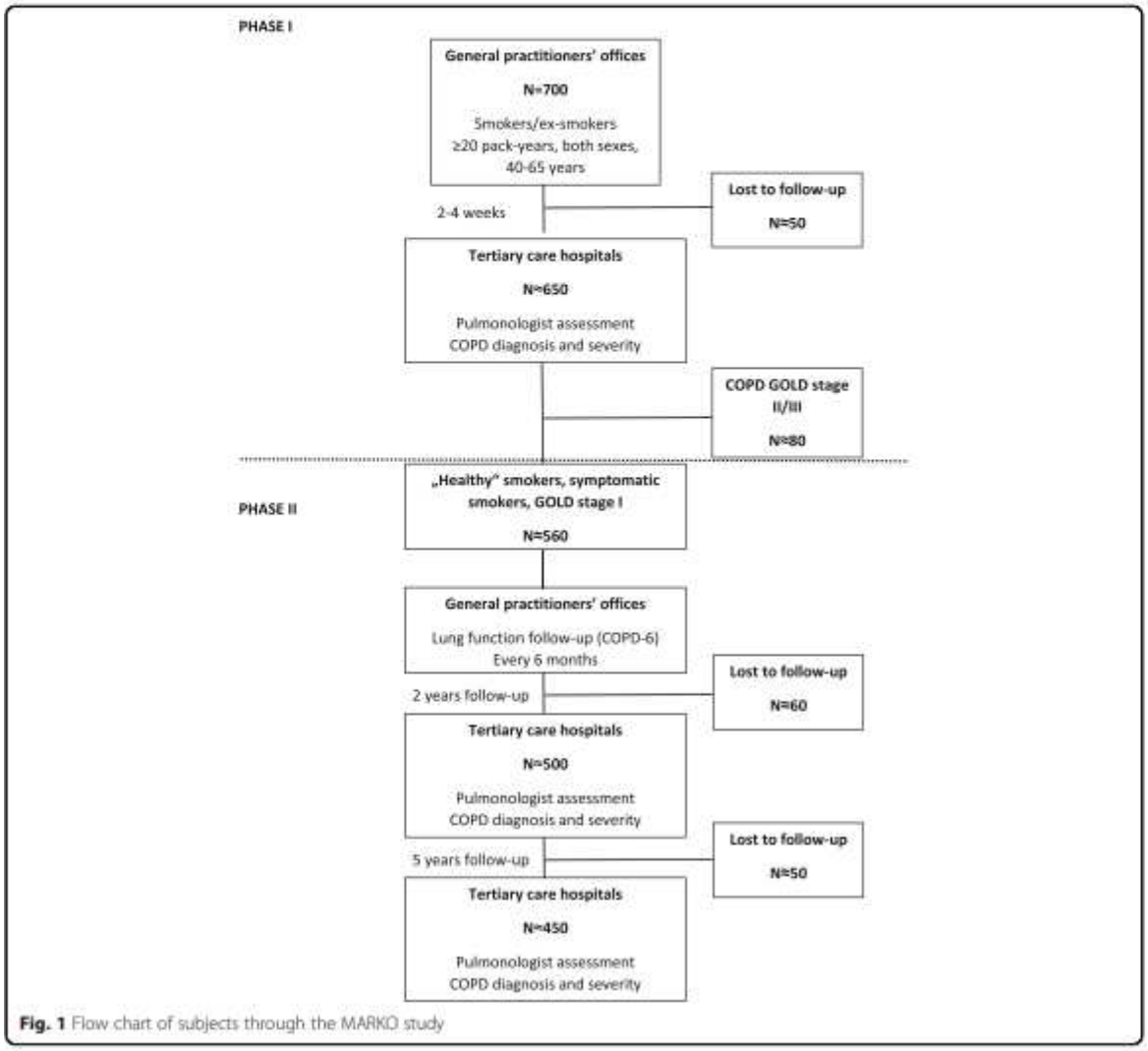

Fig. 1 Flow chart of subjects through the MARKO study

Slika 1. Protokol MARKO ispitivanja

Ispitanike (njih do 700) je bilo planirano uključiti u svakodnevnom radu liječnika obiteljske medicine (25 ordinacija inicijalno uključenih u ispitivanje). Uključili bi se pušači ili bivši pušači koji dolaze na pregled zbog problema nepovezanog s respiratornim sustavom. Uvjeti uključenja su bili: potpisani informirani pristanak, pušač/bivši pušač s >20 pušačkih godina u dobi od 40-65 godina i bez do sada postavljene dijagnoze KOPB-a. Uvjeti isključivanja iz ispitivanja su bili: bilo koja klinički značajna kronična bolest koja bi mogla utjecati na kvalitetu života ispitanika, imuno-supresivna terapija, akutna bolest dišnog sustava u posljednja 4 tjedna, hospitalizacija iz bilo kojeg razloga u posljednja 3 mjeseca, infarkt miokarda, moždani udar ili prolazna ishemijska ataka unutar 6 mjeseci, dijagnoza astme i nemogućnost provođenja nekog od predviđenih dijagnostičkih postupaka. Po uključivanju u ispitivanje, u ambulanti liječnika obiteljske medicine bi se ispunio MARKO upitnik i provelo spirometrijsko testiranje s COPD-6 priručnim spirometrom (4000 COPD-6™ Respiratory Monitor, Vitalograph Ltd., Buckingham, UK). Nakon toga bi se ispitanik unutar 2-4 tjedna uputio u jedan od

7 kliničkih centara na dalju evaluaciju. Tu bi se ponovilo ispunjavanje MARKO upitnika i provela dijagnostika plućne funkcije uz pregled pulmologa. Uz navedeno bi se ispunili i upitnici procjene kvalitete života (SGRQ i CAT). Uz spomenuto provodilo se i mjerenje temperature izdahnutog zraka prije i nakon popušene cigarete, 6-minutni test hoda i uzeta je krv za laboratorijsku obradu.

Na slici 2. prikazani su postupci s bolesnicima tijekom ispitivanja.

Table 2 List of subjects' assessment through different stages of MARKO study

Assessment	Time/place						
	0	2-4 w	6 m	12 m	18 m	24 m	60 m
	GP	P	GP	GP	GP	P	P
Prescreening	X						
Informed consent	X						
Inclusion/exclusion criteria	X						
MARKO questionnaire	X	X				X	X
CAT		X				X	X
SGRQ		X				X	X
History		X				X	X
Physical		X				X	X
EBT before and after cigarette		X					
Lung function							
COPD-6™	X	X	X	X	X		
Spirometry with bronchodilator test		X				X	X
Impulse oscilometry[a]		X					
Lung diffusion capacity		X					
Body plethysmography[a]		X					
Blood sampling (hematology; highly sensitive C-reactive protein; blood stored for DNA, RNA, plasma and serum)[b]		X					
Functional exercise capacity (6-minute walk test)		X					
COPD diagnosis		X				X	X

Legend: w - week, m - month, GP - general practitioner's office, P - pulmonologist at tertiary care hospital, CAT - COPD Assessment Test, SGRQ - St. George Respiratory Questionnaire, EBT - exhaled breath temperature
[a]Impulse oscilometry and body plethysmography was done only in one center
[b]Blood sampling was done only in subjects that signed additional Informed consent

Slika 2. Postupci tijekom ispitivanja

Očekivano je bilo od navedenog broja ispitanika izdvojiti oko 500 ispitanika s urednom plućnom funkcijom, simptomatskih pušača (prijašnji GOLD „0“) i bolesnika s KOPB GOLD „1“ stupnja koji bi se pratili tijekom 5 godina. Za to vrijeme bi se redovito kontrolirali po svojim liječnicima obiteljske medicine uz mjerenje plućne funkcije s COPD-6. Prva klinička kontrola bi bila nakon dvije godine, a druga nakon 5 godina ispitivanja. Na kliničkim kontrolama bi se definirali slijedeći bolesnici: 1. Novonastali KOPB, 2. Progresija bolesti (novonastali + pogoršanje stupnja KOPB-a) i 3. Bolesnici s ubrzanim gubitkom plućne funkcije (>70 ml/godišnje). Incidencija novo dijagnosticiranog KOPB-a nakon dvije i pet godina bi se koristila u procjeni uloge pojedinog prije mjerenog parametra u

predviđanju daljeg razvoja KOPB-a. Pohranjeni uzorci krvi od bolesnika koji su potpisali pristanak na –omics analizu bi se koristili u naknadnoj evaluaciji ovih parametara.

Ispitivanje bi trebalo dati odgovor na dva temeljna pitanja: 1. Postoji li jednostavan i jeftin način detekcije pušača/bivših pušača s rizikom za nastanak KOPB-a u populaciji s rizikom (pušači/bivši pušači) i 2. Postoji li uzorak (kombinacija) funkcionalnih parametara, kvalitete života, genetskih i biokemijskih parametara koji bi mogli otkriti bolesnika koji će razviti KOPB u skupini rizičnih ispitanika?

MARKO upitnik je izrađen u suradnji 3 iskusna pulmologa i dva psihologa. Sastoji se od 18 pitanja koja pokrivaju pojavnost i učestalost simptoma obično povezanih s KOPB-om koji mogu utjecati na kvalitetu života ispitanika. Ispitanici su pitani za tegobe unutar tri mjeseca osim za infekcije respiracijskog sustava koje su ispitivane unutar posljednjih 12 mjeseci. Uz spomenuto bilježe se i osjećaj nedostatka zraka i kondicije uz usporedbu s nepušačima iste dobi i spola. Ukupni zbroj odgovora je mogao biti od 0 do 57 gdje je veći zbroj sugerirao lošiji rezultat procjene kvalitete života.

Temperatura izdahnutog zraka je mjerena uz pomoć X-Halo® uređaja (Delmedica Investments, Singapore). Ispitanici su udisali kroz nos i izdisali kroz usta u uređaj tijekom normalnog disanja. To se ponavljalo do postizanja stabilne vrijednosti temperature izdahnutog zraka. Test se provodi na sobnoj temperaturi (19-25 °C) uz relativnu vlažnost zraka od 30–60% u laboratoriju za ispitivanje plućne funkcije. Postupak je provođen prije bilo koje druge pretrage i ponovljen najmanje 60 minuta nakon popušene cigarete (kod aktivnih pušača).

Prema našim spoznajama, ovo je prvo kohortno ispitivanje koje pokušava definirati prediktore i incidenciju KOPB-a u pre-simptomatskoj fazi bolesti i prije nastanka oštećenja plućne funkcije. Cilj istraživanja je bio razvoj jednostavnih postupaka otkrivanja KOPB-a u samom početku bolesti prije nastanka ireverzibilnog oštećenja plućne funkcije i pridruženih bolesti.

Ispitivanje je prijavljeno u registru Clinical trials.gov pod registarskim brojem NCT01550679 (https://clinicaltrials.gov/ct2/show/NCT01550679).

2.3.3. Rezultati rada „Development and the initial validation of a new self-administered questionnaire for an early detection of health status changes in smokers at risk for chronic obstructive pulmonary disease (MARKO questionnaire).“

U ovom radu je predstavljen MARKO upitnik i njegova primarna validacija u ispitivanoj skupini rizičnih pušača/bivših pušača. MARKO upitnik je razvijen od strane tri pulmologa i dva psihologa. Napisan je na hrvatskom jeziku jer je planiran za provjeru na našoj populaciji. Sastoji se od 18 pitanja i ukupni zbroj odgovora može biti od 0 do 57, s time da je veći broj povezan s izraženijim tegobama. Pojedina pitanja su redundantna s namjerom da se retroaktivno prema analizi rezultata identificiraju pitanja koja najbolje detektiraju ispitanike koji će razviti KOPB u budućnosti. MARKO upitnik je prikazan na slici 3.

MARKO

Screening Questionnaire

Identification: _____ Date of birth (MM/YYYY): _____ Sex: M W

Instructions

This questionnaire will give us the insight into your health and physical status and your breathing problems. Fill-in the questionnaire by encircling the letter in front of only one answer for each question that describes best your status.

Questions

1. Have you been coughing during preceding 3 months?
   a) No
   b) Yes, but only during cold or respiratory infection
   c) Yes, 2 or 3 times each month
   d) Yes, many times each month
   e) Almost every day

2. Have you expectorated during preceding 3 months?
   a) No
   b) Yes, but only during cold or respiratory infection
   c) Yes, 2 or 3 times each month
   d) Yes, many times each month
   e) Almost every day

3. Have you experienced being breathless during preceding 3 months?
   a) No
   b) Yes, but only during cold or respiratory infection
   c) Yes, 2 or 3 times each month
   d) Yes, many times each month
   e) Almost every day

4. Have you had a severe cold with cough or bronchitis during preceding 12 months?
   a) No
   b) 1 to 2 times
   c) 3 to 4 times
   d) 5 or more times

5. Have you used antibiotics for a severe cold with cough or bronchitis during preceding 12 months?
   a) No
   b) 1 to 2 times
   c) 3 to 4 times
   d) 5 or more times

6. How do you assess you breathing?
   a) No problems whatsoever.
   b) A little problems with breathing.
   c) Moderate problems with breathing.
   d) A lot of problems with breathing.

Do you fill breathless during these activities:

7. Sitting, lying down, rest
   a) No breathlessness
   b) Mildly breathless
   c) Moderately breathless
   d) Severely breathless

8. Everyday activities like dressing up
   a) No breathlessness
   b) Mildly breathless
   c) Moderately breathless
   d) Severely breathless

9. Walking on the same level
   a) No breathlessness
   b) Mildly breathless
   c) Moderately breathless
   d) Severely breathless

10. Climbing to the first floor level
   a) No breathlessness
   b) Mildly breathless
   c) Moderately breathless
   d) Severely breathless

1

Slika 3. MARKO upitnik

MARKO
Screening Questionnaire

Questions

11. Climbing uphill
a) No breathlessness
b) Mildly breathless
c) Moderately breathless
d) Severely breathless

12. Strenuous physical activity
a) No breathlessness
b) Mildly breathless
c) Moderately breathless
d) Severely breathless

13. Do your problems with breathing interfere with activities that you like?
a) No
b) Yes, with one or two activities
c) Yes, with several activities
d) Yes, with all activities

14. When compared with most of your peers, how do you tolerate mild exertion (e.g. walking)?
a) Better or comparable
b) Somewhat worse than most
c) Significantly worse than most
d) Much worse than most

15. When compared with most of your peers, how do you tolerate moderate exertion (e.g. sports activities)?
a) Better or comparable
b) Somewhat worse than most
c) Significantly worse than most
d) Much worse than most

16. When compared with most of your peers, how fast you become tired?
a) Slower or comparable
b) Somewhat faster than most
c) Significantly faster than most
d) Much faster than most

17. How do you rate your health?
a) Excellent
b) Very good
c) Satisfactory
d) Unsatisfactory

18. How do you rate you breathing?
a) Excellent
b) Very good
c) Satisfactory
d) Unsatisfactory

Slika 3 nastavak. MARKO upitnik

U pilot ispitivanju na 138 bolesnika s KOPB-om sva pitanja iz upitnika pokazala su visoki stupanj razumijevanja (s ocjenom >3,2 od maksimalno 4). Nakon toga je provedena inicijalna validacija upitnika koja je predviđena za procjenu osnovnih psihometrijskih karakteristika upitnika i njegovu usporedbu s postojećim upitnicima za procjenu KOPB (CAT, SGRQ). Također je bilo zanimljivo provjeriti razlikuju li se četiri ispitivane skupine (zdravi pušači, simptomatski pušači, GOLD 1 i GOLD>1) prema rezultatima MARKO upitnika. Upitnik je u dva navrata samostalno ispunjavan od strane ispitanika, prvi put u ambulanti obiteljske medicine, a drugi put prije kliničke obrade. Ispitivači

u klinici nisu imali spoznaje o rezultatima MARKO upitnika u ambulanti liječnika obiteljske medicine.

Validacija je provedena na 224 ispitanika. Većina ispitanika bili su aktivni pušači (85,8%). Muškarci su bili intenzivniji pušači (43.0 vs 32.2 PKY, P < 0.001), a kod žena je bio veći broj onih koje su prestale pušiti. Više od polovice ispitanika je imalo kronične bolesti izvan respiracijskog sustava, a skoro polovica je uzimala terapiju za neku kroničnu bolest. Kronične ili ponavljajuće respiratorne tegobe je prijavilo preko 60% ispitanika s kašljem/iskašljavanjem kod oko polovice ispitanika, a piskanja u prsima kod >20%. Karakteristike ispitanika su prikazane na slici 4.

TABLE 1. Characteristics of participants recruited in a validation study (N=224)*†

Characteristics	Total (N=224)	Men (n=110)	Women (n=114)	P
Age (years)	52.3±6.7	52.0±6.9	52.6±6.4	0.537
Smoking history (pack-years)	37.5±16.7	43.0±17.9	32.2±13.6	<0.001
Ex-smokers	32 (14.2)	22 (21.2)	10 (9.0)	0.012
Comorbidities	126 (56.3)	62 (56.4)	64 (56.1)	0.840
Chronic treatment	97 (43.3)	47 (42.7)	50 (43.9)	0.562
Body mass index (kg/m²)	26.4±4.1	27.5±3.9	25.4±4.1	<0.001
Systolic blood pressure (mmHg)	126±15	128±14	123±16	0.014
Diastolic blood pressure (mmHg)	80±9	82±9	78±9	0.003
Heart rate (min⁻¹)	80±12	79±13	80±11	0.751
Respiratory symptoms	138 (61.6)	66 (60.0)	72 (63.2)	0.627
wheezing	49 (21.9)	23 (20.9)	26 (22.8)	0.731
cough	114 (50.9)	54 (49.1)	60 (53.1)	0.550
sputum	107 (47.8)	53 (48.2)	54 (47.4)	0.903
night awakenings	16 (7.1)	10 (10.3)	6 (6.3)	0.307
chest pain	25 (11.2)	11 (10.2)	14 (12.6)	0.572
Respiratory sounds				
soft	73 (32.6)	43 (39.1)	30 (26.3)	0.027
prolonged expiration	25 (11.2)	13 (11.8)	12 (10.5)	0.949
rhonchi	30 (13.4)	15 (13.6)	15 (13.2)	0.908
Lung function (post bronchodilator)*				
FVC (% expected)	109.8±16.9	106.0±15.0	113.4±17.9	0.001
FEV_1 (% expected)	99.7±15.2	99.1±14.8	100.2±15.6	0.620
FEV_1/FVC (%)	76.0±6.4	75.2±6.7	76.8±6.0	0.066

*FVC – forced vital capacity, FEV_1 – forced expiratory volume in 1 second.
†All data are presented as mean±SD or as number (%). Statistical significance for between sex comparisons was tested using *t* test or χ^2-test.

Slika 4. Karakteristike bolesnika u validaciji MARKO upitnika

MARKO upitnik je pokazao dobru unutarnju konzistenciju i primjenjivost u uvjetima ambulante obiteljske medicine kao i pouzdanost pri samostalnom ispunjavanju od strane ispitanika. Usporedba rezultata MARKO upitnika s CAT i SGRQ upitnicima je prikazana na slici 5.

TABLE 2. Scores for the MARKO questionnaire, COPD Assessment Test (CAT) and St George Respiratory Questionnaire (SGRQ) according to different subgroups*†

		MARKO questionnaire		CAT	SGRQ scores			
		18-item	14-item	score	symptom	activity	impact	total
All (N = 224)		11 (7-18.5)	8 (3.5-13.5)	8 (4-13)	14.5 (6.3-31.8)	18.3 (6-35.5)	3.9 (0-12.4)	12.5 (4.3-21.2)
Range		0-44	0-36	0-37	0-100	0-79.6	0-55.3	0-56.5
Sex	Men (n = 110)	10 (7-17)	7 (3-13)	8 (4-12)	16.0 (4.6-30.2)	23.2 (6-32.4)	4.2 (0-14.1)	12.9 (4.4-21.8)
	Women (n = 114)	13 (6-19)	9 (4-14)	9 (4-14)	13 (7.5-34.4)	17.4 (6-35.5)	3.7 (0-9.5)	11.3 (4.1-20)
	P	0.162	0.18	0.147	0.805	0.975	0.361	0.625
Subgroups after diagnostic workup	HS (n = 72)	7 (3-11)	5 (2-9)	5 (2-8)	6.3 (0-14.2)	11.2 (0-29.3)	0 (0-4)	5.2 (1.9-13.3)
	SS (n = 110)	13 (9-20)‡	9 (4-15)‡	10 (6-15)‡	19.6 (11-37.3)‡	23.4 (6-35.6)	6.7 (0-13.4)‡	14.7 (6.8-24.1)‡
	COPD GOLD 1 (n = 23)	10 (8-20)‡	8 (5-14)	9 (4-12)	11.4 (2.6-28)	20.4 (11.2-29.5)	5.1 (0-17.2)	12.3 (3.8-21.9)
	COPD GOLD 2 (n = 19)	18 (10-26)‡	13 (7-23)‡	11.5 (6.5-18)‖	29.2 (15.1-38.7)‖	23.3 (17.4-47.7)	11.4 (3-20.8)‡	18.3 (12.1-30.1)‖
	P	<0.001	<0.001	<0.001	0.039	<0.001	<0.001	<0.001
COPD	no (n = 182)	11 (6-16)	8 (3-12)	8 (4-13)	14.1 (6.3-31.7)	17.4 (6-35.4)	3.7 (0-10.6)	11.2 (4.1-19.9)
	yes (n = 42)	14 (9-24)	10 (5.5-18.5)	9 (5-15)	22.6 (5.4-34.4)	23.3 (12.4-35.8)	7.6 (0-18)	17.2 (7.5-27)
	P	0.008	0.015	0.133	0.264	0.226	0.098	0.090
Smoking	ex-smokers (n = 32)	10 (6.5-19)	8.5 (4-13.5)	7 (3-12)	6.3 (0-16.6)	29.5 (11.8-35.6)	4.2 (0-12.9)	15.5 (4.8-21.7)
	active (n = 192)	11 (7-19)	8 (3-14)	8 (4-14)	16.6 (8.8-34.4)	17.4 (6-32.5)	3.9 (0-12.4)	12.5 (4.4-21.3)
	P	0.657	0.697	0.324	0.002	0.054	0.637	0.533
Comorbidities	no (n = 98)	10 (6-16)	7.5 (3.5-12)	8 (4-13)	14.8 (6.3-34.4)	17.4 (6-29.5)	3.8 (0-11.4)	12.6 (4.3-17.4)
	yes (n = 126)	11 (7-19)	8 (4-15)	8 (4-13)	14.1 (5.1-27.9)	23.3 (6.2-35.5)	4.2 (0-13.3)	12.3 (4.4-22)
	P	0.238	0.113	0.943	0.414	0.132	0.527	0.440
Chronic treatment	no (n = 127)	10 (6-17)	7 (3-12)	8 (4-12)	14.1 (6.3-31.7)	17.1 (6-29.5)	2 (0-10.2)	11 (3.8-17.4)
	yes (n = 97)	11 (8-19)	8 (5-15)	9 (5-14)	14.9 (6.3-34.2)	23.5 (11.2-35.6)	6.1 (0-14.3)	14.7 (5.8-22.4)
	P	0.085	0.040	0.124	0.908	0.022	0.026	0.026
Respiratory symptoms	no (n = 86)	7.5 (3-14)	6 (2-10)	5 (2-9)	6.3 (0-14.2)	11.5 (0-29.5)	0 (0-4.2)	5.2 (1.9-14.4)
	yes (n = 138)	14 (9-21)	9 (5-15)	10 (6-15)	22.5 (11.1-38)	23.3 (11.2-35.5)	7.2 (0-14.9)	15 (7.9-24.1)
	P	<0.001	<0.001	<0.001	<0.001	0.013	<0.001	<0.001
Wheezing	no (n = 175)	10 (6-15)	7 (3-12)	7 (4-11)	11 (2.3-22.4)	17.1 (6-29.5)	2 (0-9.5)	9 (3.8-17)
	yes (n = 49)	19 (13-25)	12 (9-18)	14 (10-18)	34.6 (22.6-45.8)	29.5 (18.5-41.3)	10.3 (4.3-18.1)	21.4 (14.1-27.4)
	P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Chronic/ cough sputum	no (n = 110)	9 (4-14)	6 (3-10)	7 (3-11)	8.9 (0-18.3)	17.1 (6-29.5)	0 (0-7.9)	7.6 (2.5-16.3)
	yes (n = 114)	14 (10-22)	9 (5-16)	10 (6-16)	22.9 (11.1-40.5)	23.4 (11.2-35.6)	7.4 (0-15.9)	15.7 (6.9-25.4)
	P	<0.001	<0.001	<0.001	<0.001	0.017	<0.001	<0.001
Night awakenings	no (n = 208)	11 (6-18)	8 (4-12)	8 (4-12)	12.2 (6.3-30.2)	18.2 (6.2-35.4)	3.8 (0-10.4)	12.5 (4.4-19.4)
	yes (n = 16)	23 (12-34.5)	15 (8-28.5)	17 (13-25.5)	46.9 (22.9-64.7)	26.5 (12.2-53.6)	20.9 (12.5-36.6)	27 (21.9-45.7)
	P	<0.001	0.003	<0.001	<0.001	0.076	<0.001	<0.001
Chest pain	no (n = 199)	10 (6-15)	7 (3-12)	8 (4-12)	11.9 (4.4-28)	17.4 (6-29.5)	3.7 (0-10.3)	11.1 (4.1-18.2)
	yes (n = 25)	20 (15-27)	16 (11-20)	13 (9-18)	31.7 (15.3-49.4)	41.8 (23.7-53.6)	12.2 (3.6-25.7)	25.3 (15.7-36.5)
	P	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	<0.001
Fatigue	no (n = 160)	9 (4-13)	6 (3-9)	6.5 (3-10)	11.1 (2.6-27.3)	12.2 (0-23.5)	1.8 (0-7.4)	7.7 (3.6-14.7)
	yes (n = 64)	19 (14-25)	14.5 (11-20)	13 (8-18)	21.7 (9.6-40.5)	35.8 (23.7-48)	13.7 (7.1-22.9)	23.1 (17-30.1)
	P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Soft noise on auscultation	no (n = 151)	11 (6-16)	8 (3-12)	8 (4-13)	14.1 (6.6-27.3)	17.4 (6-29.5)	3.8 (0-11.4)	11.7 (4.5-18.6)
	yes (n = 73)	11 (6-19)	8 (4-13)	8 (4-13)	11.7 (2.3-34.4)	12.4 (0-35.8)	3.9 (0-15.4)	11.1 (3.6-26)
	P	0.786	0.881	0.744	0.745	0.824	0.750	0.916
Prolonged expiration	no (n = 199)	10 (6-16)	7 (3-12)	8 (4-13)	11.6 (4.5-28)	17.4 (6-35.4)	3.9 (0-11.4)	11.2 (4-19.6)
	yes (n = 25)	16 (9-25)	10 (6-21)	9.5 (5.5-14)	21.1 (10.7-34.4)	23.7 (12.4-35.3)	7.4 (0-18.7)	17.1 (5.2-27.4)

Slika 5. Usporedba rezultata MARKO upitnika s CAT i SGRQ

TABLE 2. Continued. Scores for the MARKO questionnaire, COPD Assessment Test (CAT) and St' George Respiratory Questionnaire (SGRQ) according to different subgroups*†

		MARKO questionnaire		CAT	SGRQ scores			
		18-item	14-item	score	symptom	activity	impact	total
Rhonchi	no (n=194)	10 (6-15.5)	7 (3-12)	8 (4-12)	11.6 (3.6-27.6)	17.4 (6-35.5)	3.8 (0-10.6)	11.3 (3.8-19.6)
	yes (n=30)	18.5 (11-25)	12 (7-19)	12.5 (5.5-17)	23.8 (12.2-42.9)	18.5 (6-35.9)	10.3 (0-18.7)	13.7 (8-27.6)
	P	<0.001	0.004	0.021	0.001	0.581	0.025	0.031

*HS – "healthy" smokers/ex-smokers, SS – symptomatic smokers/ex-smokers, COPD GOLD 1 – participants diagnosed with chronic obstructive pulmonary disease (COPD) with Tiffeneau index <0.7 and forced expiratory volume in 1 second (FEV_1)>80% predicted, COPD GOLD 2 – participants diagnosed as COPD with Tiffeneau index <0.7 and FEV_1<80% and ≥50% predicted.

†All data are presented as median and interquartile range (IQR) and as range for the overall scores. Statistical significance for subgroups comparisons was tested using Mann-Whitney U test for all independent variables except for 4 subgroups according to diagnosis after the diagnostic workup that was tested using Kruskal-Wallis ANOVA.

‡Significantly different from HS (post-hoc analysis): $P < 0.05$.

§Significantly different from HS (post-hoc analysis): $P < 0.01$.

‖Significantly different from HS (post-hoc analysis): $P < 0.001$.

Slika 5 nastavak. Usporedba rezultata MARKO upitnika s CAT i SGRQ

MARKO upitnik je pokazao značajnu korelaciju s prije validiranim upitnicima za procjenu KOPB-a (SGRQ, CAT) koji se upotrebljavaju u procjeni kontrole dijagnosticirane KOPB-om. Nađena je statistički značajna korelacija s CAT upitnikom (r = 0.69, 95% CI 0.59-0.79, P < 0.001). Sa SGRQ je nađena statistički značajna umjerena korelacija s pojedinim domenama SGRQ (s domenom simptoma: r = 0.69, 95% CI 0.59-0.79, P < 0.001 i s domenom aktivnosti: r = 0.67, 95% CI 0.57-0.78, P < 0.001). Snažna pozitivna korelacija nađena je s ukupnim rezultatom SGRQ (r = 0.81, 95% CI 0.73-0.89, P < 0.001).

Kako je SGRQ složeni upitnik koji se primjenjuje u znanstvenim ispitivanjima KOPB-a, a njegova šira primjena je ograničena zahtjevnošću samog upitnika i potrebom za stručnom pomoći pri ispunjavanju, postojanje jednostavnijeg upitnika (MARKO) kojega bi bolesnik mogao samostalno ispunjavati, a koji korelira s rezultatima SGRQ moglo bi značajno olakšati znanstvena ispitivanja KOPB-a kao i redovito praćenje bolesnika u svakodnevnoj praksi.

Iako je kod svih upitnika (SGRQ, CAT i MARKO) nađena značajna varijanca između pojedinih skupina ispitanika („zdravi" pušači, simptomatski pušači, bolesnici s KOPB GOLD I i II), jedino je kod upitnika MARKO nađena statistički značajna razlika u srednjem rezultatu u usporedbi s ostale tri skupine ispitanika (M = 7 vs 13 vs 10 vs 18, P < 0.001, P = 0.045 i P < 0.001). MARKO upitnik je bolje otkrivao rane simptome kod pušača u usporedbi s ostala dva upitnika i nije pokazao značajan utjecaj pridruženih bolesti i primijenjene terapije. To upućuje na mogućnost uporabe MARKO upitnika u otkrivanju rizičnih pušača u ranoj fazi KOPB-a prije mogućnosti postavljanja dijagnoze spirometrijom.

2.3.4. Rezultati rada: „Diagnostic accuracy of a pocket screening spirometer in diagnosing chronic obstructive pulmonary disease in general practice: a cross sectional validation study using tertiary care as a reference..“

U ovom radu su prikazani rezultati usporedbe određivanja plućne funkcije priručnim spirometrom COPD-6™ (4000 COPD-6™ Respiratory Monitor, Vitalograph Ltd., Buckingham, UK) u ordinacijama liječnika obiteljske medicine i istim uređajem i standardnim spirometrom u uvjetima laboratorija za ispitivanje plućne funkcije u tercijarnim zdravstvenim ustanovama od strane educiranog osoblja. Prije provođenja ispitivanja, liječnici obiteljske medicine su prošli kratku edukaciju u malim skupinama o načinu uporabe COPD-6™ kako bi rezultati bili što sličniji rezultatima uporabe u svakodnevnoj praksi. Nakon inicijalnog mjerenja u ambulanti liječnika obiteljske medicine, unutar 2-4 tjedna bi se u uvjetima laboratorija za ispitivanje plućne funkcije tercijarne zdravstvene ustanove ponovilo mjerenje plućne funkcije s COPD-6™ i nakon toga standardnom spirometrijom s farmakodinamskim testom sa salbutamolom.

Od ukupno 326 konsekutivno odabranih pušača/bivših pušača, 227 ispitanika je uključeno u ispitivanje (185 pušača i 42 bivša pušača). Zbog isključih kriterija, 21 ispitanik nije mogao biti uključen, a 78 je odbilo dalje sudjelovanje u ispitivanju (slika 6).

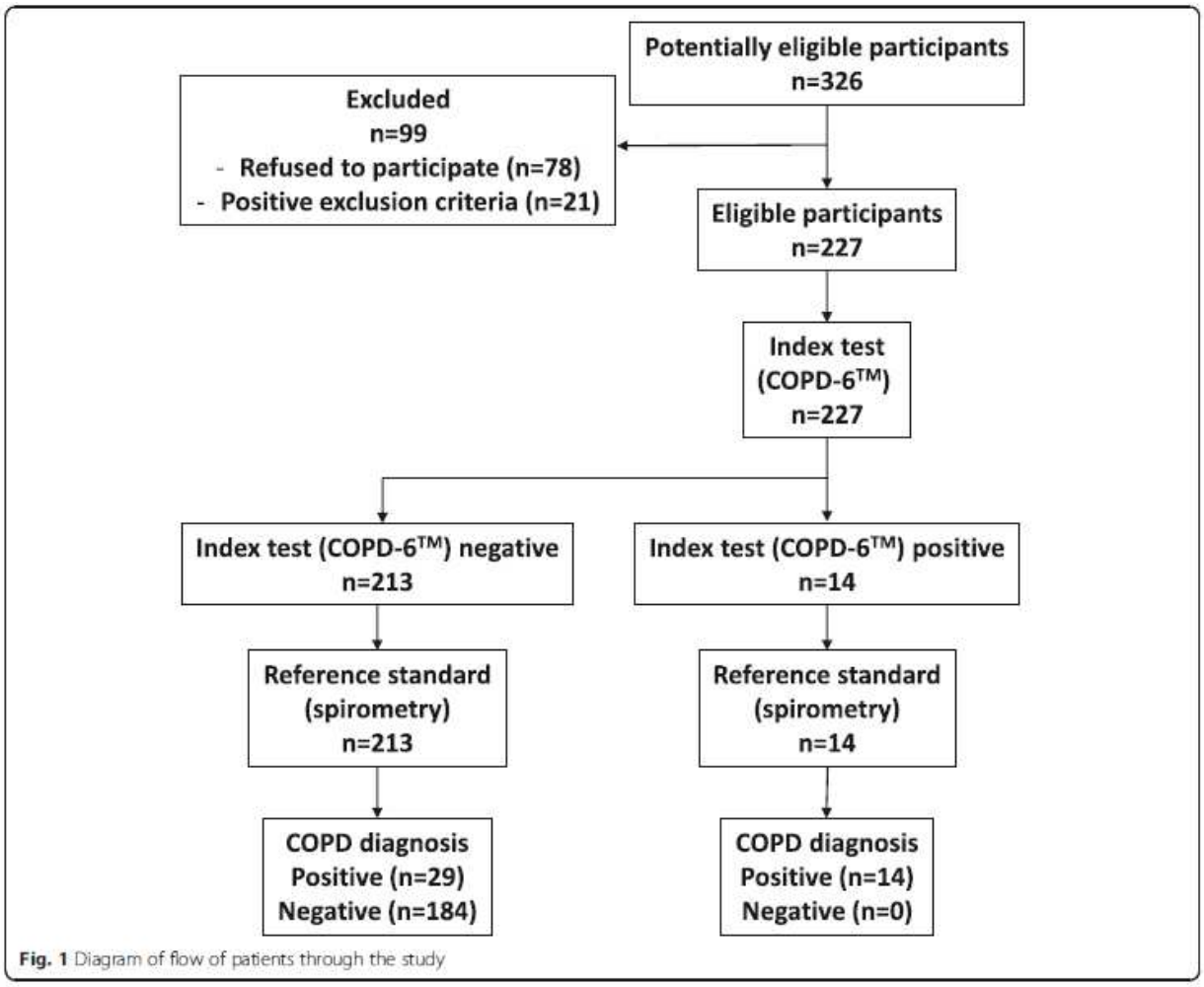

Fig. 1 Diagram of flow of patients through the study

Slika 6. Protokol ispitivanja

Demografske karakteristike, pušački status, kronične bolesti i liječenje prema postavljenoj dijagnozi KOPB-a su prikazane na slici 7.

Table 1 Demographics, smoking habit, presence of comorbid disorders and chronic treatment other than that for COPD according to final COPD diagnosis ($N = 227$)

Variables	All ($N = 227$)	COPD ($n = 43$)	Non-COPD ($n = 184$)	Statistics
Women (%)	115 (50.7)	17 (39.5)	99 (53.2)	$\chi2 = 2.711$, $P = 0.100$
Age (years), mean ± SD	52.5 ± 6.8	53.6 ± 7.0	52.3 ± 6.7	$t = 1.139$, $P = 0.256$
BMI (kgm^{-2}), mean ± SD	26.5 ± 4.2	26.5 ± 5.2	26.5 ± 3.9	$t = 0.100$, $P = 0.921$
Active smokers (%)	185 (84.9)	32 (84.2)	153 (85.0)	$\chi2 = 0.015$, $P = 0.902$
Years of smoking, mean ± SD	30.6 ± 6.9	32.0 ± 6.4	30.3 ± 6.9	$z = 1.641$, $P = 0.101$
Cigarettes/day, mean ± SD	24.6 ± 9.1	24.4 ± 8.0	24.6 ± 9.2	$z = 0.241$, $p = 0.809$
Pack-years, mean ± SD	37.9 ± 17.4	39.1 ± 14.3	37.5 ± 17.5	$z = 1.310$, $p = 0.190$
Presence of comorbid disorders (%)	126 (55.5)	22 (51.2)	104 (56.5)	$\chi2 = 0.049$, $P = 0.825$
Chronic treatment (%)	99 (43.6)	16 (37.2)	83 (45.1)	$\chi2 = 0.125$, $P = 0.724$

χ2 chi-square test results, *t* result of Student's *t*-test, *z* result of Mann-Whitney *U* test, *SD* standard deviation, *BMI* body mass index calculated as the ratio of body weight in kg and squared body height in meters

Slika 7. Demografske karakteristike, pušački status, kronične bolesti i liječenje prema postavljenoj dijagnozi KOPB-a

U našoj ispitivanoj populaciji su na ukupno 227 ispitanika otkrivena 43 bolesnika s nedijagnosticiranim KOPB-om što čini preko 18% (24 - 10.4 % GOLD stupanj 1 i 19 - 8.2 % GOLD stupanj 2. Rezultati mjerenja plućne funkcije s COPD-6 i spirometrijom su prikazani na slici 8. Analiza usporednih mjerenja prema Bland-Altmanu je prikazana na slici 9.

Table 3 Lung function (COPD-6™, spirometry) according to the presence and severity of COPD according to GOLD stages

	Lung function	All	Non-COPD	COPD GOLD 1	COPD GOLD 2	Statistics
		(*N* = 227)	(*n* = 184)	(*n* = 24)	(*n* = 19)	
COPD-6™	FEV_1 (% predicted)	94.3 ± 15.6	97.6 ± 13.3	90.9 ± 13.0	67.5 ± 12.2	$F = 46.27$ $P < 0.001$
	FEV_6 (% predicted)	93.9 ± 16.2	96.0 ± 15.3	94.7 ± 14.9	74.5 ± 13.7	$F = 17.27$ $P < 0.001$
	FEV_1/FEV_6 (%)	0.845 ± 0.085	0.864 ± 0.071	0.781 ± 0.083	0.757 ± 0.117	$F = 25.84$ $P < 0.001$
	Lung age (yrs)	60.7 ± 13.9	57.7 ± 11.1	64.0 ± 11.1	84.6 ± 15.7	$F = 48.07$ $P < 0.001$
Spirometry	FEV_1 (% predicted)	97.9 ± 15.3	101.5 ± 12.9	92.9 ± 10.2	71.1 ± 12.7	$F = 51.50$ $P < 0.001$
	FVC (% predicted)	109.3 ± 17.0	110.8 ± 16.7	112.7 ± 11.8	91.1 ± 16.2	$F = 13.29$ $P < 0.001$
	FEV_1/FVC (%)	0.742 ± 0.073	0.761 ± 0.060	0.665 ± 0.055	0.650 ± 0.073	$F = 49.14$ $P < 0.001$
	ΔFEV_1 (%)	1.39 ± 4.00	1.40 ± 3.79	2.92 ± 4.25	−0.93 ± 7.68	$F = 3.011$ $P = 0.051$

Data for all variables is presented as mean ± standard deviation; *FEV_1* forced expiratory volume in 1 s, *FEV_6* forced expiratory volume in 6 s, *FVC* forced expiratory volume, *ΔFEV_1* post-bronchodilator change in FEV_1 (measured 20 min after inhalation of 400 μg of salbutamol), F – result of ANOVA for between group comparisons

Slika 8. Rezultati mjerenja plućne funkcije s COPD-6 i spirometrijom stratificirani prema dijagnozi.

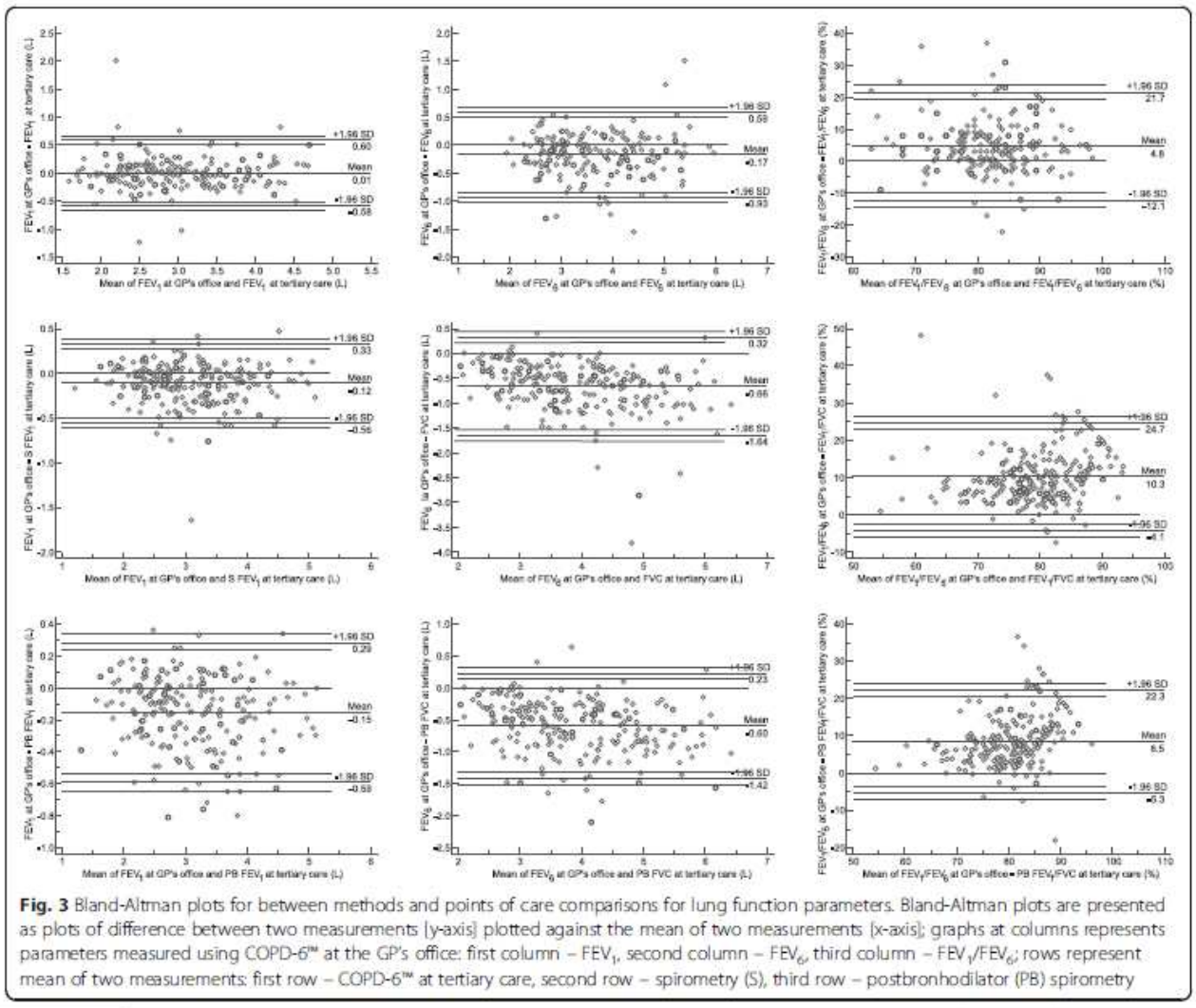

Fig. 3 Bland-Altman plots for between methods and points of care comparisons for lung function parameters. Bland-Altman plots are presented as plots of difference between two measurements (y-axis) plotted against the mean of two measurements (x-axis); graphs at columns represents parameters measured using COPD-6™ at the GP's office: first column – FEV_1, second column – FEV_6, third column – FEV_1/FEV_6; rows represent mean of two measurements: first row – COPD-6™ at tertiary care, second row – spirometry (S), third row – postbronhodilator (PB) spirometry

Slika 9. Analiza usporednih mjerenja prema Bland-Altmanu

Mjerenje plućne funkcije priručnim COPD6 spirometrom u ordinaciji liječnika obiteljske medicine pokazalo je umjerenu pouzdanost s visokom specifičnošću, ali s niskom osjetljivosti za otkrivanje KOPB-a. Uređaj se može pouzdano koristiti u ordinaciji liječnika obiteljske medicine uz rezultate usporedive s onima postignutim u kliničkim uvjetima što čini razliku prema cjelovitoj spirometriji. Također, veliki dio bolesnika se može klasificirati prema tim rezultatima te se može smatrati kako omjer FEV1/FEV6 >0,85 s velikom sigurnosti može isključiti postojanje KOPB-a dok se omjer FEV1/FEV6 <0,7 može smatrati potvrdom dijagnoze KOPB-a te bi se samo rizični bolesnici s omjernom FEV1/FEV6 0,7-0,85 trebali dalje dijagnostički obrađivati što bi značajno pojednostavilo dijagnostiku KOPB-a i omogućilo otkrivanje oboljelih u rizičnoj skupini te ranije započinjanje liječenja. Na temelju naših rezultata može se preporučiti algoritam aktivnog traženja bolesnika s KOPB-om u rizičnoj populaciji koji je prikazan na slici 10.

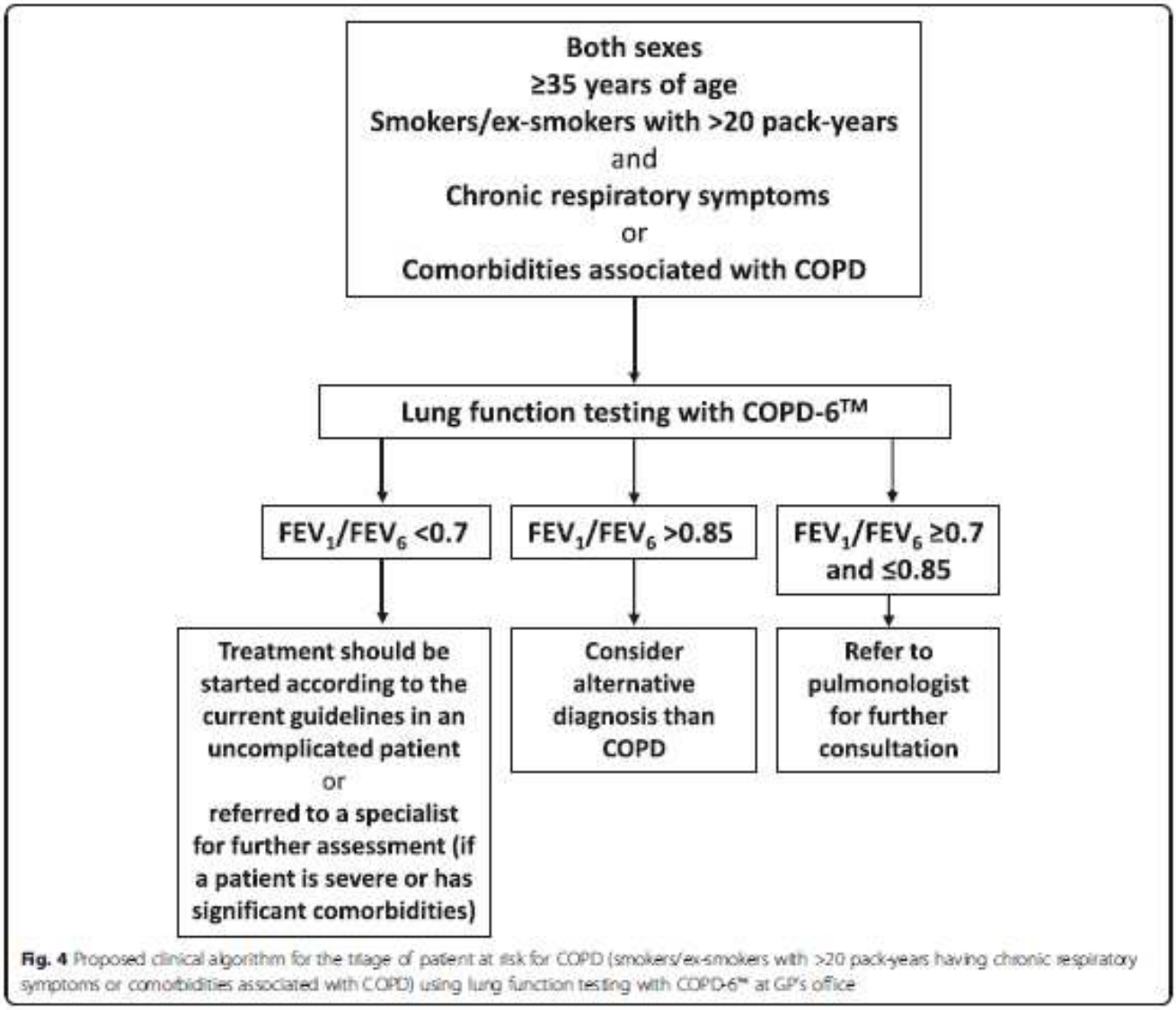

Fig. 4 Proposed clinical algorithm for the triage of patient at risk for COPD (smokers/ex-smokers with >20 pack-years having chronic respiratory symptoms or comorbidities associated with COPD) using lung function testing with COPD-6™ at GP's office

Slika 10. Preporučeni algoritam aktivnog traženja bolesnika s KOPB-om u rizičnoj populaciji.

2.3.5. Rezultati rada: „.Exhaled Breath Temperature as a Novel Marker of Future Development of COPD: Results of a Follow-Up Study in Smokers.“

U ovom radu je naglašen značaj mjerenja temperature izdahnutog zraka kao biljega aktivnosti upalnog procesa u KOPB-u. Kako samo oko 1/3 pušača razvije KOPB tijekom života, od velikog značaja bi bilo definirati biljege koji bi u populaciji pušača mogli otkriti one koji su „osjetljivi“ na pušenje i kod kojih će ono dovesti do nastanka KOPB-a. Unatoč brojnim ispitivanjima, do sada nije definiran takav biljeg. Kronični upalni odgovor pluća na unošenje štetnih čestica je jedan od osnovnih patofizioloških mehanizama nastanka KOPB-a. Do danas nije definiran način mjerenja intenziteta tog odgovora. Mjerenje temperature izdahnutog zraka se u drugim bolestima pluća pokazalo kao mogući parametar aktivnosti upalnog odgovora u plućima. U našem ispitivanju smo na populaciji „zdravih“ pušača koristili mjerenje temperature izdahnutog zraka prije i nakon popušene cigarete kao mjeru aktivnosti upalnog odgovora na popušenu cigaretu. Iz cjelokupne populacije u ispitivanju su izdvojeni aktivni pušači (jer je ispitivanje podrazumijevalo pušenje jedne cigarete tijekom ispitivanja). Ukupno je uključeno 140 ispitanika. Kod njih je u sklopu planirane obrade učinjeno i mjerenje temperature izdahnutog zraka prije svih drugih planiranih postupaka i najmanje sat vremena nakon posljednje popušene cigarete te nakon 15 minuta od popušene jedne cigarete. Isti bolesnici su ponovno obrađivani nakon dvije godine i praćena je progresija u novonastalom KOPB-u, progresija stupnja KOPB-a ili stupanj pogoršanja plućne funkcije (>70 ml/godišnje). Mjerenje temperature izdahnutog zraka je provođeno prema prije definiranim metodama uz pomoć X-Halo® uređaja (Delmedica Investments, Singapore). Promjena u temperaturi izdahnutog zraka se bilježila kao apsolutna razlika dviju izmjerenih vrijednosti.

Rezultati usporedbe promjene temperature izdahnutog zraka i stupnja KOPB-a nakon dvije godine (novodijagnosticirani KOPB, progresije bolesti, ubrzani gubitak plućne funkcije) su prikazani na slici 11.

Table 4. EBT (EBTb, EBTc and ΔEBT) ROC curve analysis data according to the outcomes of disease progression after 2-year follow-up (N = 140).

Disease progression outcome			EBTb	EBTc	ΔEBT
ND COPD	GOLD	AUC, 95% CI, Statistics	0.664, 0.571–0.748, Z = 1.199, p = 0.231	0.568, 0.466–0.667, Z = 0.547, p = 0.584	0.669, 0.568–0.759, Z = 1.409, p = 0.159
	LLN	AUC, 95% CI, Statistics	0.788, 0.703–0.858, **Z = 2.330, p = 0.020**	0.704, 0.605–0.791, Z = 1.124, p = 0.261	0.859, 0.781–0.917, **Z = 2.533, p = 0.011**
DP	GOLD	AUC, 95% CI, Statistics	0.663, 0.579–0.740, Z = 1.595, p = 0.111	0.530, 0.438–0.621, Z = 0.274, p = 0.784	0.711, 0.622–0.789, **Z = 2.404, p = 0.016**
	LLN	AUC, 95% CI, Statistics	0.716, 0.634–0.789, **Z = 2.246, p = 0.025**	0.731, 0.644–0.808, **Z = 1.993, p = 0.046**	0.614, 0.520–0.702, Z = 0.783, p = 0.433

Legend: ND COPD – newly diagnosed COPD after 2 years of follow-up; DP - disease progression – ND COPD + progression of a severity of COPD from GOLD 1 stage during the 2-year follow-up; GOLD: Global Initiative for Chronic Obstructive Lung Disease; LLN: lower level of normal; AUC: area under the curve; CI: confidence interval; EBT – exhaled breath temperature; EBTb – baseline EBT; EBTc – EBT after smoking cigarette; ΔEBT – change in EBT after smoking cigarette; statistical significance was tested using z statistics.

Slika 11. Rezultati usporedbe promjene temperature izdahnutog zraka i stupnja KOPB-a nakon dvije godine (Novo-dijagnosticirani KOPB, progresije bolesti, ubrzani gubitak plućne funkcije).

Razlika u temperaturi izdahnutog zraka od 1°C je imala graničnu prediktivnu vrijednost za predviđanje nastanka novootkrivenog KOPB-a (rezultat je bio značajniji ukoliko su se koristili ATS/ERS kriteriji prema donjoj granici referentnih vrijednosti - LLN). Za progresiju bolesti je promjena temperature izdahnutog zraka od 0,17°C imala statistički značajnu prediktivnu vrijednost i kod procjene prema GOLD smjernicama i kod primjene ATS/ERS preporuka. Nismo našli statistički značajne prediktivne vrijednosti promjene temperature izdahnutog zraka na ubrzano propadanje plućne funkcije. Visoka negativna prediktivna vrijednost (>95%) upućuje na veliku dozu pouzdanosti u negativan rezultat kao mjerodavan u procjeni „osjetljivosti“ pušača na udisanje duhanskog dima.

U ovom radu smo dokazali povezanost promjene temperature izdahnutog zraka prije i nakon pušenja cigarete sa stupnjem aktivnosti KOPB-a i time definirali promjenu temperature izdahnutog zraka kao jedan od prvih bio-markera osjetljivosti na štetne učinke duhanskog dima u rizičnoj populaciji što bi trebalo provjeriti na većem uzorku kroz dulje vrijeme praćenja.

2.3.6. Rezultati rada: „Breathlessness and “exacerbation” questions predictive for incident COPD (MARKO study): data after two years of follow-up.“

U ovom radu smo usporedili rezultate inicijalnog ispitivanja i kontrole nakon dvije godine. Od 366 ukupno regrutiranih „zdravih“ pušača/bivših pušača i simptomatskih pušača/bivših pušača, na kontrolni pregled je došlo 320 ispitanika (od čega 186 žena). Novo-dijagnosticirani KOPB je nađen kod 33 ispitanika (stopa incidencije 4,911/100 pacijent-godina). KOPB se razvio statistički značajno češće kod muškaraca (75.8% vs 37.3%, $p < 0.001$) i kod ispitanika s većom izloženosti duhanskom dimu (42.99 vs 35.52 pušačkih godina, $p = 0.008$). Kod ispitanika koji su razvili KOPB inicijalno se nalazio veći stupanj opstrukcije (FEV1, Tiffeneau indeks, MEF25 i MEF50), DLco je bio usporediv između dvije skupine ali je KCO bio inicijalno značajno niži u KOPB-ovoj skupini. Šestominutni test hoda, mMRC, hematološki nalazi i visoko osjetljivi CRP nisu pokazali razlike između dvije skupine. Nije nađeno razlika niti u ukupnim rezultatima SGRQ, MARKO i CAT upitnika. Značajna razlika je nađena u odgovoru na 4. pitanje MARKO upitnika (ataka kašlja i bronhitisa u proteklih 12 mjeseci) uz marginalnu značajnost za pitanja 3. (zaduha u posljednja tri mjeseca) i 5. (uporaba antibiotika u posljednjih 12 mjeseci).

Usporedba rezultata između dvije skupine je prikazana na slici 12.

Table 3 **Comparison of baseline measures between the groups of incident COPD and rest (at follow-up visit) (*N* = 320).**

Variable	No COPD (*n* = 287)		Incident COPD (*n* = 33)				
	Mean	SD	Mean	SD	Difference	95% CI	*p*[a]
Age (yrs)	51.76	7.38	52.38	7.80	0.62	[−2.06 to 3.31]	0.561
Sex (male)	106	37.3%	25	75.8%			<0.001
Smoking history (p/y)	35.52	17.01	42.99	19.18	7.47	[1.23–13.71]	0.008
Time from baseline (yrs)	2.11	0.21	2.14	0.22	0.03	[−0.05 to 0.11]	0.483
Body height (cm)	171.08	9.25	177.76	9.46	6.68	[3.32–10.04]	<0.001
Body weight (kg)	78.45	15.92	84.24	17.37	5.79	[−0.03 to 11.61]	0.046
BMI (kgm^{-2})	26.69	4.32	26.52	4.45	−0.17	[−1.74 to 1.40]	0.841
Heart rate (min^{-1})	77.76	12.26	76.79	13.22	−0.97	[−5.80 to 3.85]	0.788
Systolic blood pressure (mmHg)	127.26	14.50	131.43	12.39	4.17	[−1.42 to 9.76]	0.123
Diastolic blood pressure (mmHg)	80.38	9.07	80.57	9.08	0.19	[−3.35 to 3.73]	0.893
Commorbidities (No)	0.69	0.78	0.64	0.86	−0.05	[−0.34 to 0.24]	0.599
Chronic treatments (No)	0.86	1.13	0.82	1.26	−0.04	[−0.45 to 0.38]	0.591
Post BD FVC (L)	4.04	1.00	4.66	1.15	0.62	[0.25–0.99]	0.003
Post BD FVC (% predicted)	97.74	12.83	97.47	14.01	−0.26	[−5.03 to 4.50]	0.760
Post BD FVC (z score)	−0.17	0.92	−0.19	1.05	−0.02	[−0.36 to 0.33]	0.749
Post BD FEV1 (L)	3.20	0.80	3.13	0.85	−0.07	[−0.37 to 0.22]	0.795
Post BD FEV1 (% predicted)	97.95	13.94	83.35	15.11	−14.60	[−19.77 to −9.43]	<0.001
Post BD FEV1 (z score)	−0.14	1.02	−1.21	1.12	−1.07	[−1.45 to −0.69]	<0.001
Post BD TIFF (%)	79.51	5.62	67.21	5.55	−12.30	[−14.33 to −10.27]	<0.001
Post BD TIFF (% predicted)	99.81	6.89	85.24	7.60	−14.58	[−17.10 to −12.05]	<0.001
Post BD TIFF (z score)	0.01	0.86	−1.67	0.83	−1.68	[−1.99 to −1.37]	<0.001
Post BD MEF25 (L/s)	1.21	0.58	0.79	0.35	−0.42	[−0.63 to −0.20]	<0.001
Post BD MEF50 (L/s)	4.02	1.43	2.53	1.22	−1.50	[−2.02 to −0.97]	<0.001

Slika 12. Usporedba rezultata pojedinih parametara nakon dvije godine ispitivanja između skupine koja nije razvila KOPB i ispitanika koji su razvili KOPB.

Table 3 Comparison of baseline measures between the groups of incident COPD and rest (at follow-up visit) (N = 320).

Variable	No COPD (n = 287)		Incident COPD (n = 33)				
	Mean	SD	Mean	SD	Difference	95% CI	p[a]
Post BD PEF (L/s)	7.66	2.08	8.01	2.18	0.35	[−0.43 to 1.13]	0.199
DLCO (% predicted)	78.16	18.26	75.53	33.23	−2.63	[−10.76 to 5.50]	0.083
KCO (% predicted)	79.30	19.39	71.31	23.64	−7.99	[−16.02 to 0.04]	0.049
6 MWT (m)	442.39	88.85	433.81	100.29	−8.57	[−44.38 to 27.24]	0.763
6 MWT (%)	63.28	11.77	58.25	13.59	−5.03	[−9.79 to −0.28]	0.056
EBTb (°C)	33.01	2.83	32.43	3.37	−0.58	[−1.70 to 0.53]	0.404
EBTd (°C)	−0.06	1.46	0.40	1.92	0.46	[−0.16 to 1.08]	0.521
RBC	4.69	0.42	4.71	0.41	0.03	[−0.14 to 0.20]	0.626
Hgb	142.27	13.12	145.96	12.98	3.69	[−1.73 to 9.12]	0.208
htc	0.43	0.05	0.43	0.03	0.01	[−0.01 to 0.03]	0.179
WBC	8.26	1.97	8.10	2.04	−0.16	[−0.98 to 0.66]	0.639
hsCRP	3.32	4.03	2.80	2.15	−0.51	[−2.20 to 1.17]	0.769
mMRC	0.67	0.77	0.73	0.84	0.06	[−0.22 to 0.34]	0.770
SGRQ activity score	23.36	20.00	25.71	18.62	2.35	[−5.06 to 9.76]	0.463
SGRQ impact score	7.66	10.66	9.56	10.43	1.91	[−2.06 to 5.88]	0.136
SGRQ symptom score	19.54	18.42	25.79	23.60	6.25	[−0.84 to 13.34]	0.290
SGRQ total score	14.42	12.63	17.15	12.80	2.73	[−1.99 to 7.45]	0.163
CAT (score)	9.46	6.85	12.07	7.77	2.61	[−0.01 to 5.23]	0.052
MQq 1	1.40	1.35	1.91	1.55	0.50	[0.00–1.01]	0.099
MQq 2	1.22	1.32	1.59	1.52	0.37	[−0.12 to 0.87]	0.207
MQq 3	0.63	1.00	1.06	1.32	0.44	[0.06–0.82]	0.064
MQq 4	0.42	0.54	0.66	0.55	0.24	[0.04–0.43]	0.015
MQq 5	0.31	0.49	0.50	0.57	0.19	[0.00–0.37]	0.050
MQq 6	0.63	0.63	0.75	0.67	0.12	[−0.12 to 0.35]	0.331
MQq 7	0.27	0.58	0.28	0.58	0.02	[−0.20 to 0.23]	0.799
MQq 8	0.25	0.53	0.25	0.51	0.00	[−0.20 to 0.19]	0.951
MQq 9	0.24	0.51	0.25	0.44	0.01	[−0.17 to 0.20]	0.574
MQq 10	0.56	0.73	0.69	0.97	0.13	[−0.15 to 0.40]	0.751
MQq 11	1.19	0.91	1.28	0.89	0.10	[−0.24 to 0.43]	0.602
MQq 12	1.30	0.96	1.38	0.79	0.07	[−0.28 to 0.42]	0.604
MQq 13	0.41	0.73	0.56	0.80	0.15	[−0.12 to 0.42]	0.148
MQq 14	0.33	0.62	0.31	0.47	−0.02	[−0.25 to 0.20]	0.730
MQq 15	0.57	0.73	0.63	0.71	0.05	[−0.21 to 0.32]	0.546
MQq 16	0.41	0.70	0.41	0.61	0.00	[−0.26 to 0.25]	0.774
MQq 17	1.42	0.78	1.34	0.65	−0.08	[−0.36 to 0.20]	0.387
MQq 18	1.32	0.80	1.38	0.71	0.05	[−0.23 to 0.34]	0.900
MQ (total score)	12.89	8.89	15.22	10.32	2.33	[−1.00 to 5.65]	0.277

Notes:
[a] Mann-Whitney test.
p/y , pack-years; BMI, body mass index; HR, heart rate; BD, bronchodilator; FVC, forced vital capacity; FEV1, forced expiratory volume in 1 s; TIFF, Tiffeneau index (FEV1/FVC); MEF25, mid-expiratory flow at 75% of FVC; MEF50, mid-expiratory flow at 50% of FVC; PEF, peak expiratory flow; DLCO, diffusing capacity of the lungs for carbon monoxide; KCO, carbon monoxide transfer coefficient; 6MWT, 6-min walk test; EBTb, baseline exhale breath temperature; EBTd, difference in exhaled breath temperature after smoking a cigarette; RBC, red blood cell count; Hgb, hemoglobin; htc, hematocrit; WBC, white blood cell count; hsCRP, high-sensitivity C-reactive protein; mMRC, modified Medical Research Council dyspnea scale; CAT, COPD Assessment Test; SGRQ, St. George's respiratory questionnaire; MQ, MARKO questionnaire; MQq, MARKO questionnaire question number.

Slika 12. nastavak

Analizom uz korištenje multi varijantne logističke regresije nađeno je kako su pitanja o zadusi, egzacerbacijama i muški spol mogli predvidjeti povećani rizik za nastanak KOPB-a unutar dvije godine. Rezultati analize su prikazani na slici 13.

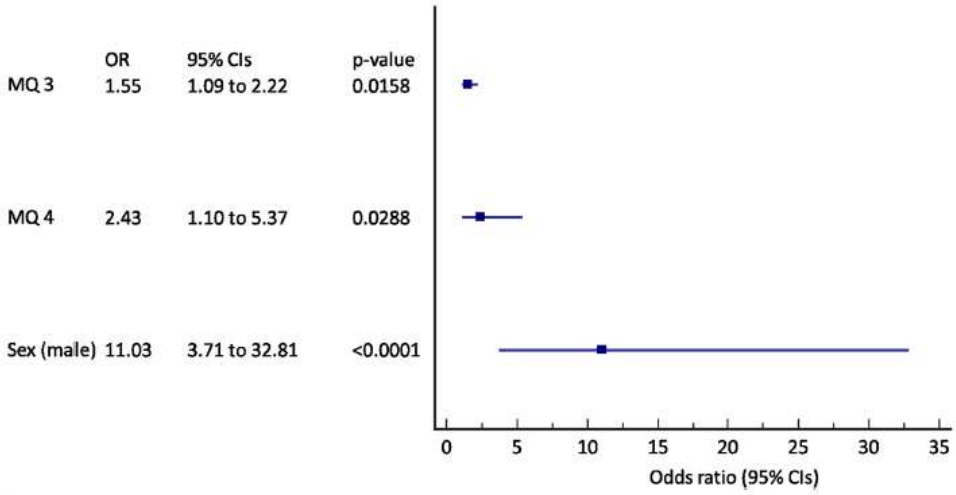

Figure 2 Forest plot of the results of the multivariate logistic regression for the prediction of incident COPD (*N* = 320). MQ, MARKO questionnaire question number; CIs, confidence intervals. Full-size DOI: 10.7717/peerj.16650/fig-2

Slika 13. Forest plot prikaz rezultata analize multivarijantne logističke regresije za predviđanje incidencije KOPB-a

Kod pušača kod kojih je ispitivana i razlika temperature izdahnutog zraka prije i nakon popušene cigarete, za svako povećanje razlike za 0,01ºC je došlo do povećanja rizika za 29% (slika 14.)

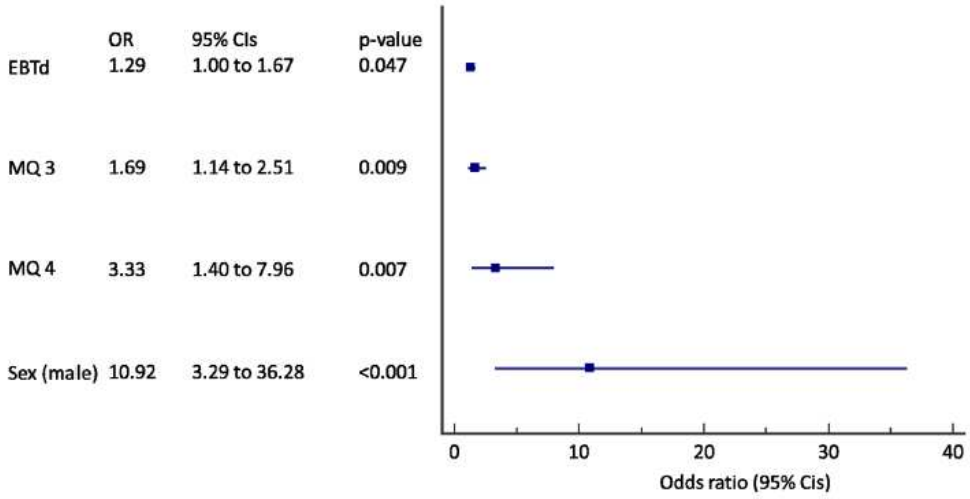

Figure 3 Forest plot of the results of the multivariate logistic regression for the prediction of incident COPD in active smokers (N = 245). EBTd, change in exhaled breath temperature after a smoked cigarette; MQ, MARKO questionnaire question number; CIs, confidence intervals.

Slika 14. Forest plot prikaz rezultata analize multi varijantne logističke regresije za predviđanje incidencije KOPB-a kod pušača

Ovim rezultatima smo otvorili novo područje istraživanja značaja neregistriranih/blagih pogoršanja KOPB-a kod bolesnika koji još ne zadovoljavaju spirometrijske kriterije za KOPB u razvoju i progresiji bolesti. Pokazali smo da rizični ispitanici koji imaju učestalu potrebu za liječenjem respiracijskih infekcija s antibioticima imaju veći rizik nastanka KOPB-a od onih koji nisu imali takve probleme. Također smo potvrdili značaj mjerenja temperature izdahnutog zraka u praćenju aktivnosti KOPB-a i verificirali moguću ulogu praćenja razlike temperature izdahnutog zraka prije i nakon inhalacije duhanskog dima u svrhu predviđanja rizika za razvoj KOPB-a u rizičnoj populaciji.

2.4. RASPRAVA

KOPB je jedan od vodećih uzroka morbiditeta i mortaliteta u svijetu. Ukoliko želimo smanjiti morbiditet i mortalitet od KOPB-a, potrebno je postići napredak u sljedećim koracima (24,25):

1. Prevencija: Kampanje protiv pušenja, edukacija, prevencija pušenja u mlađoj populaciji, zamjena uporabe goriva od biomase u kućanstvu, smanjenje zagađenja ispušnim plinovima u prometu, promocija zdravog razvoja i očuvanja pluća.

2. Rana dijagnoza: Otkrivanje bolesnika u ranoj fazi KOPB-a uz aktivno pronalaženje oboljelih u populaciji pod rizikom.

3. Potrebno je uspostaviti registre osoba koje su pod povećanim rizikom za nastanak KOPB-a i definirati protokole njihovog praćenja.

4. Dijagnosticirati pred-klinički KOPB: Definirati i otkriti bolesnike s patofiziološkim procesom koji će dovesti do trajnog oštećenja plućne funkcije. Odrediti bio-markere i druge pokazatelje koji bi mogli otkriti KOPB u toj fazi, a prije nastanka spirometrijskog poremećaja. Striktno praćenje takvih bolesnika uz provođenje mjera za prestanak pušenja / izloženosti štetnim čimbenicima.

5. Otkriti terapijske pristupe koji će biti učinkoviti u sprječavanju daljeg oštećenja pluća i razvoja KOPB-a iz pre-kliničke faze.

Naše ispitivanje je fokusirano u smjeru ranog otkrivanja postojećeg KOPB-a i mogućnosti dijagnostike pre-kliničkog KOPB-a. Za osobe iz rizičnih skupina postoji veći broj različitih preporuka za rano otkrivanje oboljelih (2). GOLD preporuča aktivno traženje oboljelih unutar rizične populacije, ali ne preporuča spirometrijski probir. Jedan od razloga kasnog otkrivanja oboljelih je smanjena dostupnost spirometrije, osobito u ruralnim sredinama i mjestima udaljenim od kliničkih centara. Zbog toga se razmatra nekoliko strategija. Jedna je provođenje spirometrije na razini obiteljske medicine, ali to zahtijeva veliki broj spirometara i značajan stupanj edukacije izvršitelja te je u ispitivanjima pokazalo sub-optimalne rezultate (26). Drugi pristup je primjena priručnih jednostavnih spirometara koji mjere ograničeni dio plućne funkcije. Problem s njima je nedovoljna podudarnost rezultata dobivenih tim mjerenjem i standardne spirometrije (26,27). U našem istraživanju smo dokazali postojanje značajne potrebe za aktivnijim nalaženjem oboljelih od KOPB-a u rizičnoj populaciji jer je novootkriveni KOPB nađen kod skoro 20% ispitanika iz rizične skupine. Što se tiče postavljanja dijagnoze putem priručnog spirometra, rezultati su bili slični ispitivanjima drugih takvih uređaja, ali smo analizom rezultata preporučili novi pristup uporabi takvih uređaja. Naime umjesto

insistiranja na 100% podudarnosti sa zlatnim standardom dijagnostike KOPB-a (spirometrijom u laboratoriju za analizu plućne funkcije), priručnim spirometrima se može učiniti dodatni probir u populaciji pod rizikom koristeći dvije umjesto jedne granične vrijednosti. Na taj način smo u našem ispitivanju mogli s jednom vrijednosti isključiti mogućnost postojanja KOPB-a, a s drugom je potvrditi čime bi se značajno smanjio broj bolesnika koji nisu mogli biti dijagnosticirani u ambulanti obiteljske medicine i kod kojih je trebalo za konačnu dijagnozu provesti punu spirometrijsku obradu. Prema našem mišljenju, na ovaj način bi se mogao značajno povećati broj bolesnika s KOPB-om dijagnosticiranih u ranoj fazi bolesti od strane liječnika obiteljske medicine.

Kako se već i kod rane KOPB-a nalazi značajno ireverzibilno oštećenje plućne funkcije, sve više se preporuča otkrivanje bolesnika s aktivnim patofiziološkim procesom koji će dovesti do nastanka KOPB-a i započeti terapijsku intervenciju već u toj fazi. Navedeno stanje se naziva pre-KOPB, ali za sada još nije jasno definirano niti postoje jednoznačne preporuke kako ga otkriti i kako postupiti s takvim bolesnicima (28-30). Jedna od preporuka je praćenje simptoma i parametara kvalitete života bolesnika jer se njihovo pogoršanje povezuje s aktivnosti patofiziološkog procesa i povećanim rizikom za nastanak KOPB-a (31,32). Kako je većina upitnika za praćenje kvalitete života u KOPB-u kompleksna i neprilagođena redovitom praćenju bolesnika izvan kliničkih ispitivanja, mi smo u našem radu formirali novi, jednostavniji upitnik usmjeren na samoprocjenu simptoma i stanja povezanih s ranim KOPB-om. Testiranjem MARKO upitnika na našoj populaciji dokazali smo dobru korelaciju s postojećim upitnicima za procjenu KOPB-a, a neki elementi MARKO upitnika su bili statistički značajno povezani s povećanim rizikom za nastanak KOPB-a u rizičnoj populaciji. To su dominantno pitanja iz domene zaduhe i učestalosti respiracijskih infekcija uz bronhitične smetnje i uporaba antibiotika za njihovo liječenje. Ovaj rezultat upućuje na značaj dvije domene koje se koriste u procjeni KOPB-a (zaduha i egzacerbacije) i prije nastanka samog KOPB-a. Znajući kako najveći stupanj propadanja plućne funkcije nastaje u ranim fazama KOPB-a i kako su egzacerbacije značajan čimbenik daljeg pogoršanja plućne funkcije, ovi rezultati impliciraju potrebu za boljim definiranjem pojma egzacerbacije KOPB-a, osobito pitanje detekcije i značaja blagih egzacerbacija. Prema našim rezultatima, pušači skloni učestalim respiracijskim infekcijama bi mogli imati povećan rizik nastanka oštećenja plućne funkcije i samog KOPB-a.

Kako do 50% pušača ima neke tegobe, ali samo kod oko 8% njih dođe do nastanka KOPB-a, potrebni su nam mehanizmi dodatnog razdvajanja tih ispitanika. Upalni odgovor kao reakcija na unošenje štetnih čestica u pluća se smatra jednim od osnovnih patofiziološkim mehanizama nastanka oštećenja plućne funkcije kod pušača (33-35). Brojni parametri upalnog procesa su ispitivani u cilju ranog otkrivanja patofizioloških promjena koje dovode do KOPB-a, ali većina njih nije pokazala

zadovoljavajući dijagnostički potencijal, najvjerojatnije jer se radi o niskom intenzitetu upale i procesu dugog trajanja (34,35). Trenutno se ispituje mogućnost praćenja upalnih biljega u kondenzatu izdahnutog zraka u cilju mjerenja intenziteta upale i prije nastanka KOPB-a. Mi smo u našem radu ispitivali promjenu temperature izdahnutog zraka kao biljeg upalne aktivnosti na unošenje štetnih čestica (duhanskog dima) u pluća pušača (36,37). Do povećanja temperature izdahnutog zraka nakon pušenja cigarete ne dolazi kod svih pušača pa bi se ova činjenica mogla koristiti u razlikovanju pušača osjetljivih na posljedice pušenja i onih kod kojih neće doći do oštećenja sukladnog s KOPB-om. Korelacija povećanja temperature izdahnutog zraka nakon pušenja cigarete i povećanog rizika za nastanak KOPB-a nakon dvije godine praćenja koju smo našli u našem istraživanju upućuje na mogućnost uporabe mjerena temperature izdahnutog zraka kao jeftinog, jednostavnog i dostupnog parametra otkrivanja pojedinaca kod kojih pušenje izaziva aktivaciju upalnog odgovora u plućima, a time i dovodi do promjena povezanih s nastankom KOPB-a.

Iako je naše istraživanje dalo dosta zanimljivih rezultata i smjernica za dalje ispitivanje te smo dokazali barem ograničenu mogućnost predviđanja nastanka KOPB-a u rizičnoj populaciji na temelju praćenja jednostavnih parametara, postoje i određena ograničenja. Uzorak ispitanika je srazmjerno mali i očekivana incidencija KOPB-a nije dostignuta. Kako smo ispitivali jednostavne biljege koji bi mogli predvidjeti nastanak KOPB-a, nismo pratili druge moguće opcije (genetika, epigenetika, metabolomika) koji su mogli dati dodatnu vrijednost u analizi, ali smo za određeni broj bolesnika sačuvali biološki materijal (serum) za moguću naknadnu analizu. Također nismo imali kontrolnu skupinu nepušača iste dobi i spola. Navedena ograničenja su nastala uslijed ograničenog budžeta projekta. Rezultati našeg istraživanja bi se trebali provjeriti i potvrditi usporedivim ispitivanjima na većoj populaciji ispitanika s rizikom za nastanak KOPB-a.

2.5. ZAKLJUČCI

Projekt MARKO je pokrenut s idejom rješavanja dva osnovna problema u liječenju KOPB-a, a to je što ranije postavljanje dijagnoze KOPB-a u populaciji i otkrivanje bolesnika s danas definiranim terminom pre-KOPB-a, naime pušača s povećanim rizikom za razvoj KOPB-a. Uz navedeno, u projektu je praćen veći broj parametara i uzeti su uzorci za moguću naknadnu analizu bioloških parametara koji bi mogli upućivati na početne patofiziološke promjene koje će u daljem tijeku rezultirati nastankom KOPB-a. Primarni rezultati MARKO projekta kao i analiza ispitanika nakon dvije godine praćenja ukazali su na nove smjerove kojima bi trebalo ići otkrivanje ranog KOPB-a kao i definiranju bolesnika s povećanim rizikom koji bi se trebali intenzivnije pratiti i započeti liječenje, u prvom redu prestanak pušenja, prije nastanka ireverzibilnih promjena vezanih uz KOPB. Za podrobnije definiranje uporabe navedenih rezultata u svakodnevnoj kliničkoj praksi potrebno je provesti ispitivanje na većem broju ispitanika.

2.6. SAŽETAK

Uvod: Pušenje je glavni uzrok nastanka KOPB-a, ali samo oko 1/3 pušača razviju KOPB tijekom života. Progresivni tijek KOPB-a uz značajan morbiditet i mortalitet indiciraju potrebu za što ranijim otkrivanjem bolesti kako bi se što prije započelo s liječenjem.

Metode: MARKO projekt je multicentrično prospektivno kohortno istraživanje koje uključuje 500 ispitanika s rizikom za nastanak KOPB-a (pušači/bivši pušači ≥20 pušačkih godina u dobi od 40-65 godina, oba spola, bez prije postavljene dijagnoze KOPB-a). Ispitivanje je planirano u dvije faze: (1) presječno istraživanje: razvoj i validacija novog upitnika uz evaluaciju učinkovitosti ručnog COPD-6™ spirometra u ordinaciji obiteljske medicine u pronalaženju bolesnika s KOPB-om u rizičnoj populaciji; i (2) prospektivno istraživanje: praćenje kohorte ispitanika iz populacije pod rizikom za nastanak KOPB-a. Ispitanici su uključeni iz 25 ambulanti obiteljske medicine i pregledani od strane pulmologa u 7 bolničkih centara prema predefiniranom protokolu: upitnicima za s bolesti povezanom kvalitetom života, anamnezom, fizikalnim pregledom, uzimanjem uzoraka krvi, mjerenjem temperature izdahnutog zraka, mjerenjem plućne funkcije i šestominutnim testom hoda. Ispitanici kod kojih nije postavljena dijagnoza KOPB-a kao i oni s KOPB-om prvog stupnja po GOLD-u su uključeni u dalje praćenje i procjenu progresije bolesti nakon dvije godine, a planira se i ponovna evaluacija nakon 5 godina praćenja.

Rezultati: U prvoj fazi, KOPB je dijagnosticiran kod 43 ispitanika (18,9% od ispitanika s rizikom) što indicira poboljšanje postupaka za pronalaženje oboljelih unutar populacije s rizikom za KOPB. Portabilni COPD-6™ spirometar je pokazao visoku specifičnost i nisku osjetljivost za postavljanje dijagnoze KOPB-a, ali smo dokazali kako se uporabom dvije različite granične vrijednosti ispitanici mogu podijeliti u dvije skupine: skupinu s dokazanim KOPB-om (FEV1/FEV6 < 0.7) i skupinu kod koje je isključeno postojanje KOPB-a (FEV1/FEV6>0.85) što ostavlja samo manji broj nedefiniranih bolesnika između ove dvije vrijednosti, a kod kojih je indicirana dalja dijagnostička obrada. Na temelju psihometrijske analize i visoke podudarnosti s validiranim upitnicima za procjenu kvalitete života, MARKO upitnik pokazao se pouzdanim kao kratki alat za samoprocjenu ispitanika.

U drugoj fazi nakon dvije godine praćenja, našli smo učestalost novonastalog KOPB-a od 4.911/100 osoba-godina (95% CI [3.436–6.816]). Analizom eksploratornih čimbenika MARKO upitnika izolirane su tri odvojene domene (zaduha i umor, "egzacerbacije", kašalj uz iskašljavanje). Pitanja o zadusi i egzacerbacijama kao i muški spol su bili prediktivni čimbenici za nastanak KOPB-a u rizičnoj populaciji nakon dvije godine praćenja (AUC 0.79, 95% CI [0.74–0.84], p < 0.001). Promjena temperature izdahnutog zraka nakon pušenja cigarete (ΔEBT) pri inicijalnoj obradi je bila značajno

prediktivna za progresiju bolesti ($p<0.05$) uz AUC od 0.859 ($p=0.011$) osjetljivost od 66.7% i specifičnost od 98.1% za novo dijagnosticirani KOPB uz uporabu LLN kriterija.

Zaključak: Ovo je jedno od prvih kohortnih ispitivanja koje pokušava ustanoviti incidenciju KOPB-a kod pre-simptomatskih ispitanika iz rizične skupine prije nastanka značajnog oštećenja ciljnih organa. Rezultati našeg ispitivanja upućuju na potrebu uvođenja aktivnog traženja oboljelih u populaciji s rizikom (nađeno je skoro 20% nedijagnosticiranih bolesnika s KOPB-om). Testiranje plućne funkcije s COPD-6™ može zamijeniti spirometriju ukoliko ona nije lako dostupna. Rezultati MARKO upitnika u kombinaciji s ΔEBT mogu potencijalno poslužiti kao rani biljeg budućeg razvoja KOPB-a kod rizičnih pušača.

Registracija ispitivanja: Clinicaltrial.gov NCT01550679 retrospektivno registrirano 28. veljače 2012.

2.7. SUMMARY

Introduction: Main risk factor for the development of chronic obstructive pulmonary disease (COPD) is smoking, but only less than 1/3 of smokers develop clinically manifest COPD. COPD's progressive nature with high disability and mortality makes it plausible to detect it as early as possible thus allowing for an early intervention.

Methods: MARKO project is a multicenter prospective cohort study recruiting 500 subjects at risk for COPD (smokers/ex-smokers ≥20 pack-years, 40–65 years, both sexes, with no prior diagnosis of COPD) in two phases: (1) cross-sectional: development and validation of a new questionnaire and evaluation of the efficacy of the handheld COPD-6™ in GP's office in case finding of COPD from the population at risk; and (2) prospective: follow-up of a cohort of patients at risk for COPD. Subjects were recruited by 25 GPs and assessed for COPD by dedicated pulmonologists in 7 hospital centers using a predefined protocol: HRQoL, history, physical, blood sampling, exhaled breath temperature (EBT), lung function, 6-min walk test (6MWT). Patients without COPD and those in GOLD stage 1 at initial assessment were reassessed for disease progression by the same pulmonologist after 2 years and are planned to be reassessed again after 5 years.

Results: In the phase I, COPD was diagnosed in 43 subjects (18.9 %), which indicate the need for better case finding procedures in the population at risk. The handheld COPD-6™ showed high specificity but low sensitivity for the COPD diagnosis but we found that by using two different cut-off values we can stratify the majority of the patients to the two groups: COPD group (FEV1/FEV6 < 0.7) and group without COPD (FEV1/FEV6>0.85) leaving only small number of patients between these two values for further diagnostic workout. Based on psychometric analyses and high convergent validity correlation with already validated QoL questionnaires, the newly developed MARKO questionnaire was shown to be a reliable self-administered short health status assessment tool.

In the phase II, after two years of follow up, we have determined a rate for incident COPD that was 4.911/100 person-years (95% CI [3.436–6.816]). Exploratory factor analysis of MARKO questionnaire isolated three distinct domains (breathlessness and fatigue, "exacerbations", cough, and expectorations). We found out that questions about breathlessness and "exacerbations", and male sex were predictive of incident COPD after two years follow-up (AUC 0.79, 95% CI [0.74–0.84], $p < 0.001$). The change in EBT after smoking a cigarette at initial visit (ΔEBT) was significantly predictive for disease progression after 2 years ($p<0.05$) with an AUC of 0.859 ($p=0.011$) with sensitivity of 66.7% and specificity of 98.1% for newly diagnosed COPD using LLN criteria.

Conclusion: This is one of the first cohort studies attempting to establish the incidence of COPD in the presymptomatic stage before significant end organ damage. Our study results point out that active case finding in a population at risk for COPD should be instituted (almost 20 % of undiagnosed COPD). Based on our results lung function testing with COPD-6™ can substitute spirometry testing in cases where it is not readily available. The MARKO questionnaire combined with ΔEBT could potentially serve as early markers of future COPD in smokers at risk.

Trial registration: Clinicaltrial.gov NCT01550679 retrospectively registered February 28, 2012.

2.8. LITERATURA

1. GBD 2015 Chronic Respiratory Disease Collaborators. Global, regional, and national deaths, prevalence, disability-adjusted life years, and years lived with disability for chronic obstructive pulmonary disease and asthma, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet Respir Med. 2017;5(9):691-706
2. 2024 GLOBAL STRATEGY FOR PREVENTION, DIAGNOSIS AND MANAGEMENT OF COPD. https://goldcopd.org/ GOLD-2024_v1.1-1Dec2023_WMV.pdf assessed: 05DEC2023
3. WHO website: https://www.who.int/respiratory/copd/burden/en/ assessed 05DEC2020
4. Raherison C, Girodet PO. Epidemiology of COPD. Eur Respir Rev. 2009 Dec;18(114):213-21. doi: 10.1183/09059180.00003609. PMID: 20956146
5. Allen JL. Airway function throughout the lifespan: Pediatric origins of adult respiratory disease. Pediatr Investig. 2019 Dec 21;3(4):236-244. doi: 10.1002/ped4.12165. PMID: 32851329; PMCID: PMC7331336
6. Wang G, Kull I, Bergström A, Hallberg J, Bergström PU, Guerra S et al. Early-life risk factors for reversible and irreversible airflow limitation in young adults: findings from the BAMSE birth cohort. Thorax. 2020 Nov 12:thoraxjnl-2020-215884. doi: 10.1136/thoraxjnl-2020-215884. Epub ahead of print. PMID: 33184098
7. Billatos E, Ash SY, Duan F, Xu K, Romanoff J, Marques H, DECAMP and COPDGene Investigators. Distinguishing Smoking-Related Lung Disease Phenotypes Via Imaging and Molecular Features. Chest. 2020 Sep 16:S0012-3692(20)34494-9. doi: 10.1016/j.chest.2020.08.2115. Epub ahead of print. PMID: 32946850
8. Choi JY, Rhee CK. Diagnosis and Treatment of Early Chronic Obstructive Lung Disease (COPD). J Clin Med. 2020 Oct 26;9(11):3426. doi: 10.3390/jcm9113426. PMID: 33114502; PMCID: PMC7692717
9. Fazleen A, Wilkinson T. Early COPD: current evidence for diagnosis and management. Ther Adv Respir Dis. 2020 Jan-Dec;14:1753466620942128. doi: 10.1177/1753466620942128. PMID: 32664818; PMCID: PMC7394029
10. Soriano JB, Polverino F, Cosio BG. What is early COPD and why is it important? Eur Respir J. 2018 Dec 6;52(6):1801448. doi: 10.1183/13993003.01448-2018. PMID: 30309976

11. Welte T, Vogelmeier C, Papi A. COPD: early diagnosis and treatment to slow disease progression. Int J Clin Pract. 2015 Mar;69(3):336-49. doi: 10.1111/ijcp.12522. Epub 2014 Oct 31. PMID: 25363328
12. Han MK, Ye W, Kim DY, Woodruff P; Pulmonary Trials Cooperative Investigators. Design of the Redefining Therapy in Early COPD Study. Chronic Obstr Pulm Dis. 2020 Oct;7(4):382-389. doi: 10.15326/jcopdf.7.4.2020.0157. PMID: 32989941
13. Ritchie AI, Martinez FJ. The Challenges of Defining Early COPD in the General Population. Am J Respir Crit Care Med. 2020 Dec 3. doi: 10.1164/rccm.202011-4176ED. Epub ahead of print. PMID: 33271056
14. Laucho-Contreras ME, Cohen-Todd M. Early diagnosis of COPD: myth or a true perspective. Eur Respir Rev. 2020 Dec 2;29(158):200131. doi: 10.1183/16000617.0131-2020. PMID: 33268437
15. Han MK, Agusti A, Celli BR, Criner GJ, Halpin DG, Roche N, et al. From Gold 0 to Pre-COPD. Am J Respir Crit Care Med. 2020 Nov 19. doi: 10.1164/rccm.202008-3328PP. Epub ahead of print. PMID: 33211970
16. Şerifoğlu İ, Ulubay G. The methods other than spirometry in the early diagnosis of COPD. Tuberk Toraks. 2019 Mar;67(1):63-70. English. doi: 10.5578/tt.68162. PMID: 31130137
17. Siafakas N, Bizymi N, Mathioudakis A, Corlateanu A. EARLY versus MILD Chronic Obstructive Pulmonary Disease (COPD). Respir Med. 2018 Jul;140:127-131. doi: 10.1016/j.rmed.2018.06.007. Epub 2018 Jun 5. PMID: 29957274
18. Xiao T, Wu K, Chen Y, Qiu H, Ruan X, Wang N et al. Quality of life and its associated factors for mild chronic obstructive pulmonary disease patients of urban community settings. Ann Palliat Med. 2020 Jul;9(4):1420-1430. doi: 10.21037/apm-19-655. Epub 2020 Jul 14. PMID: 32692206
19. Danov O, Wolff M, Bartel S, Böhlen S, Obernolte H, Wronski S et al. Cigarette Smoke Affects Dendritic Cell Populations, Epithelial Barrier Function, and the Immune Response to Viral Infection With H1N1. Front Med (Lausanne). 2020 Nov 6;7:571003. doi: 10.3389/fmed.2020.571003. PMID: 33240904; PMCID: PMC7678748
20. Lee YJ, Choi S, Kwon SY, Lee Y, Lee JK, Heo EY et al. A Genome-Wide Association Study in Early COPD: Identification of One Major Susceptibility Loci. Int J Chron Obstruct Pulmon Dis. 2020 Nov 17;15:2967-2975. doi: 10.2147/COPD.S269263. PMID: 33235445; PMCID: PMC7680157
21. Kwon DS, Choi YJ, Kim TH, Byun MK, Cho JH, Kim HJ, Park HJ. FEF25-75% Values in Patients with Normal Lung Function Can Predict the Development of Chronic Obstructive

Pulmonary Disease. Int J Chron Obstruct Pulmon Dis. 2020 Nov 12;15:2913-2921. doi: 10.2147/COPD.S261732. PMID: 33209020; PMCID: PMC7669499

22. Cosío BG, Pascual-Guardia S, Borras-Santos A, Peces-Barba G, Santos S, Vigil L et al. Phenotypic characterisation of early COPD: a prospective case-control study. ERJ Open Res. 2020 Oct 5;6(4):00047-2020. doi: 10.1183/23120541.00047-2020. PMID: 33043045; PMCID: PMC7533304
23. Popov TA, Kralimarkova TZ, Labor M, Plavec D. The added value of exhaled breath temperature in respiratory medicine. J Breath Res. 2017 Aug 8;11(3):034001. doi: 10.1088/1752-7163/aa7801
24. Plavec D, Vrbica Ž. What is pre-COPD and do we know how to treat it? Expert Rev Respir Med. 2024 Jul 3:1-6. doi: 10.1080/17476348.2024.2375418
25. Stolz D, Mkorombindo T, Schumann DM, et al. Towards the elimination of chronic obstructive pulmonary disease: a lancet commission. The Lancet. 2022 Sep 17;400(10356):921–972. doi: 10.1016/S0140-6736(22)01273-915
26. Zhou J, Li X, Wang X, Yu N, Wang W. Accuracy of portable spirometers in the diagnosis of chronic obstructive pulmonary disease A meta-analysis. NPJ Prim Care Respir Med. 2022 Apr 19;32(1):15. doi: 10.1038/s41533-022-00275-x. PMID: 35440665; PMCID: PMC9019105
27. Kim B-S, Park S-H, Jung S-S, Kim H-J, Woo S-D, Lee M-M. Validity Study for Clinical Use of Hand-Held Spirometer in Patients with Chronic Obstructive Pulmonary Disease. Healthcare. 2024; 12(5):507. doi:10.3390/healthcare12050507
28. Wijnant SRA, De Roos E, Kavousi M, et al. Trajectory and mortality of preserved ratio impaired spirometry: the Rotterdam study. Eur Respir J. 2020;55:1901217
29. Grant T, Brigham EP, McCormack MC. Childhood origins of adult lung disease as opportunities for prevention. J Allergy Clin Immunol Pract. 2020;8(3):849–858. doi: 10.1016/j.jaip.2020.01.015
30. Çolak Y, Nordestgaard BG, Vestbo J, et al. Prognostic significance of chronic respiratory symptoms in individuals with normal spirometry. Eur Respir J. 2019;54(3):1900734. doi: 10.1183/ 13993003.00734-2019
31. Woodruff PG, Barr RG, Bleecker E, et al. SPIROMICS Research Group. Clinical significance of symptoms in smokers with preserved pulmonary function. N Engl J Med. 2016;374(19):1811–1821. doi: 10. 1056/NEJMoa1505971

32. Colak Y, Afzal S, Nordestgaard BG, et al. Prognosis of asymptomatic and symptomatic, undiagnosed COPD in the general population in Denmark: a prospective cohort study. Lancet Respir Med. 2017;5 (5):426–434. doi: 10.1016/S2213-2600(17)30119-417
33. Valavanidis A, Vlachogianni T, Fiotakis K. Tobacco smoke: involvement of reactive oxygen species and stable free radicals in mechanisms of oxidative damage, carcinogenesis and synergistic effects with other respirable particles. Int J Environ Res Public Health. 2009 Feb;6(2):445–462. doi: 10.3390/ijerph6020445
34. Wang Y, Liao J, Zhong Y, et al. Predictive value of combining inflammatory biomarkers and rapid decline of FEV(1) for COPD in Chinese population: a prospective cohort study. Int J Chronic Obstruct Pulm Dis. 2019;14:2825–2833. doi: 10.2147/COPD.S223869
35. Zemans RL, Jacobson S, Keene J, et al. Multiple biomarkers predict disease severity, progression and mortality in COPD. Respir Res. 2017;18(1):117. doi: 10.1186/s12931-017-0597-727
36. Lee J, Taneja V, Vassallo R. Cigarette smoking and inflammation: cellular and molecular mechanisms. J Dent Res. 2012;91 (2):142–149. doi: 10.1177/0022034511421200
37. Popov TA, Kralimarkova TZ, Labor M, et al. The added value of exhaled breath temperature in respiratory medicine. J Breath Res. 2017 Aug 8;11(3):034001. doi: 10.1088/1752-7163/aa7801

3. PRESLIKE RADOVA

3.1. Early detection of COPD patients in GOLD 0 population: an observational non-interventional cohort study - MARKO study.

Vrbica et al. BMC Pulmonary Medicine (2017) 17:36
DOI 10.1186/s12890-017-0378-6

BMC Pulmonary Medicine

STUDY PROTOCOL Open Access

Early detection of COPD patients in GOLD 0 population: an observational non-interventional cohort study - MARKO study

Žarko Vrbica[1,2†], Marina Labor[3,4†], Ivan Gudelj[5], Slavica Labor[3,4], Iva Jurić[6], Davor Plavec[4,7*] and for the MARKO study group

Abstract

Background: Main risk factor for the development of chronic obstructive pulmonary disease (COPD) is smoking, although only less than 1/3 of smokers develop clinically manifest COPD. COPD's progressive nature with high disability and mortality makes it plausible to detect it as early as possible thus allowing for an early intervention. The only tool for an early diagnosis that could be used on the global scale is spirometry, even though symptoms and deprivation of health related quality of life (HRQoL) precede relevant spirometric changes. Existing HRQoL questionnaires are too complicated or not developed for an early detection of COPD. The aim of our study was to develop a new simple HRQoL tool that will allow (alone or in combination with other markers) early detection of patients with COPD.

Methods: A multicenter prospective cohort study recruiting 500 subjects at risk for COPD (smokers/ex-smokers ≥ 20 pack-years, 40–65 years, both sexes, with no prior diagnosis of COPD) will be carried out in two phases: (1) cross-sectional - development and validation of a new questionnaire; and (2) prospective - follow-up of a cohort of patients at risk for COPD. Subjects were recruited by 25 GPs and assessed for COPD by dedicated pulmonologists in 7 hospital centers using a predefined protocol: HRQoL, history, physical, blood sampling, exhaled breath temperature (EBT), lung function, 6-min walk test (6MWT). Patients without COPD and those in GOLD stage 1 at initial assessment will be reassessed for disease progression by the same pulmonologist after 2 and 5 years.

Discussion: This is one of the first cohort studies attempting to establish the incidence of COPD in the pre-symptomatic stage before significant end organ damage. We intend to assess the validity, predictability and discriminative power ('healthy' smokers vs. pre-symptomatic phase in newly developed COPD) of newly developed HRQoL tool alone or in combination with other markers; EBT, lung function, 6MWT, genomics, transcriptomics, proteomics). We expect that the results of this study can improve our understanding of the development of COPD, identify some new underlying pathophysiological pathways, and offer to sensitive smokers/ex-smokers new preventive and early intervention measures thus improving the management of COPD.

Trial registration: Clinicaltrial.gov NCT01550679 retrospectively registered February 28, 2012.

Keywords: Biological markers, Chronic obstructive pulmonary disease (COPD), Cigarette smoking, Disease susceptibility, Early diagnosis

* Correspondence: plavec@bolnica-srebrnjak.hr
†Equal contributors
[4]Faculty of Medicine, J.J. Strossmayer University of Osijek, Ulica cara Hadrijana 10E, Osijek, Croatia
[7]Research Department, Children's Hospital Srebrnjak, Srebrnjak 100, Zagreb, Croatia
Full list of author information is available at the end of the article

Background

Chronic obstructive pulmonary disease (COPD) is a major cause of morbidity and mortality globally, responsible for approximately 5 million deaths every year, with expected significant rise in mortality untill the year 2020. COPD mortality is low at age 45, but significantly rises after the age of 65 with significant comorbidity already present. COPD has a major economical and public health impact especially with regard to severe disease and exacerbations. Major risk factor for COPD development is tobacco smoke, although only less than 1/3 of smokers develop COPD during their lifetime. Because of its progressive nature COPD ends with early disability and mortality. Stopping or slowing down the progression of the disease is still an unmet need, although therapeutic interventions in COPD patients have shown to have significantly larger impact if they were started earlier in the course of the disease [1–4]. An early diagnosis should allow for an early intervention, thus possibly preventing the progression of COPD, alleviating symptoms and improving general wellbeing, preventing complications and comorbidities and early mortality [5]. The only available method for an early diagnosis that is suitable for screening purposes is spirometry (also used as the 'gold standard' for COPD diagnosis). Although screening on a global scale is not recommended by COPD guidelines, recommendations for early diagnosis (case finding) have been heavily advocated [6, 7]. New insights of the genetic background of COPD development have been revealed from cross-sectional large scale genetic studies but these data still need confirmation on a global scale using prospective data [8]. On the other hand, symptoms and loss in health related quality of life (HRQoL) often precede diagnostically relevant loss of lung function (FEV_1/FVC <0.7 or < lower limit of normal [LLN]) [6]. Consequently we need new simple tools to detect patients in an early (pre)symptomatic stage of COPD before significant end organ damage. Based on these assumptions; the possibility of identifying the population at risk and the possibility of producing significant benefit for the patient if COPD is identified early, we decided to develop and test a new tool (to be used alone and in combination with other markers) in a prospective cohort study in a population at risk (smokers/ex-smokers with significant cumulative exposure to tobacco smoke, with no prior diagnosis of COPD). This new tool should be based on a prerequisite for a screening tool; usable on a global level, cheap, self-applicable, moderate to high sensitivity and high specificity (no false positives) for COPD. As existing HRQoL questionnaires are too complicated (Chronic Respiratory Questionnaire - CRQ, St. George Respiratory Questionnaire - SGRQ), and/or not developed for the purpose of early detection of COPD (Clinical COPD Questionnaire - CCQ, COPD Assessment Test - CAT), we have constructed, developed, and intend to validate a simple self-applicable questionnaire to detect early changes in HRQoL related to future COPD development [9–13].

Methods

The aim, design and setting of the study

The aim of this two phase prospective observational cohort study in subjects at risk for COPD was to construct, develop and validate a new tool (newly constructed self-applicable HRQoL questionnaire – MARKO questionnaire) to be used alone or in combination with other markers (exhaled breath temperature [EBT], lung function, inflammatory markers, and omics) in order to identify subjects who will develop COPD, even before significant end organ damage that can be identified using spirometry.

The MARKO study is a prospective, observational, non-interventional cohort study of subjects (both sexes) at risk for the development of COPD (smokers/ex-smokers with a smoking history of ≥20 pack-years), without a previous diagnosis of COPD. The study for all investigational sites was approved by the Children's Hospital Srebrnjak Ethics Committee and performed in accordance with the Declaration of Helsinki, good clinical practice, and all relevant international and national legislations. According to a national legislation regarding non-interventional studies an approval from a single local ethics committee meets the ethical requirements for such a study. The MARKO study has been carried out with the cooperation of 25 general practitioners (GPs) and 7 tertiary hospital research centers (within Departments of Pulmonology) since 2010 (Table 1).

Primary endpoint: to develop and validate the MARKO questionnaire to be used alone or together with other markers to identify subjects that will develop COPD.

Secondary endpoints: (a) to evaluate psychometric characteristics of the MARKO questionnaire; (b) to evaluate if the developed screening questionnaire together with the COPD-6™ measurement discriminates COPD patients with GOLD stage 0 (symptomatic smokers) and GOLD 1 from 'healthy' smokers and patients with COPD GOLD 2 or higher based on the evaluation made by the pulmonologist; (c) to determine the rate of progression of COPD in patients with GOLD 0 during the follow-up; (d) to determine the prevalence of concomitant disorders in this population; (e) to identify diagnostic parameters that are most sensitive for early impairment in COPD (best discriminate between COPD GOLD 0 and GOLD 1); (f) to compare the MARKO questionnaire with diagnostic tools used for evaluation of patients (SGRQ, CAT, history, physical, lung function, 6MWT); (g) to assess the prevalence of different stages of COPD (specifically

Table 1 The list of study sites and researchers involved in MARKO study (MARKO study group)

Study sites	Contact person/researcher	Role
Children's Hospital Srebrnjak, Zagreb, Croatia	Assoc.Prof. Davor Plavec, MD, MSc, PhD	Principal Investigator
General Hospital Dubrovnik, Dubrovnik, Croatia	Žarko Vrbica, MD, MSc	Principal Investigator
University of Liverpool, Liverpool, UK	Prof. Peter MA Calverley, MD, PhD	Study consultant
University of Zagreb Centre for Croatian Studies, Zagreb, Croatia	Assist.Prof. Adrijana Koščec Đuknić, PhD	Psychologist
Institute for Medical Research and Occupational Health, Zagreb, Croatia	Assist.Prof. Biserka Radošević-Vidaček, BA, MA, PhD	Psychologist
University Hospital Center Osijek, Osijek, Croatia	Marina Labor, MD, PhD	Co-Investigator
	Slavica Labor, MD, PhD	Subinvestigator
	Iva Jurić, MD	Subinvestigator
University Hospital Center Split, Split, Croatia	Assist.Prof. Ivan Gudelj, MD, PhD	Co-Investigator
University Hospital Center Rijeka, Rijeka, Croatia	Assist.Prof. Ljiljana Bulat Kardum, MD, PhD	Co-Investigator
University Hospital Dubrava, Zagreb, Croatia	Assoc.Prof. Neven Tudorić, MD, PhD	Co-Investigator
	Đivo Ljubičić, MD	Subinvestigator
University Hospital Center Zagreb, Zagreb, Croatia	Assoc.Prof. Sanja Popović Grle, MD, PhD	Subinvestigator
Special Hospital for Lung Diseases, Zagreb, Croatia	Tajana Jalušić Glunčić, MD	Subinvestigator
	Tina Lukić, MD	Subinvestigator
General practice offices, Osijek, Croatia	Albina Dumić, MD, PhD	
	Renata Girgurić, MD	
	Ljiljana Ismić, MD	
	Monika Jeđud, MD	
	Jasna Nagyszombaty-Šarić, MD	
	Darja Nelken-Bestvina, MD	
	Sanda Pribić, MD	
	Alen Stojanović, MD	
	Bojana Vakanjac, MD	
General Practice offices, Split, Croatia	Merim Bezdrov, MD	
	Mirjana Bezdrov, MD	
	Ivana Boban, MD	
	Jadranka Radman, MD	
	Rosanda Rosandić Plasevoli, MD	
	Vanja Viali, MD	
General Practice offices, Zagreb, Croatia	Vjekoslava Ameri-Šakić, MD	
	Ljiljana Lulić-Karapetrić, MD	
	Suzana Maltar-Delija, MD	
	Dubravka Margaretić, MD	
	Davorka Martinković, MD	
	Zdenka Meštrović, MD	
General Practice office, Donji Muć, Croatia	Karla Tudja, MD	
General Practice office, Postira, Croatia	Nataša Mrduljaš-Đujić, MD	
General practice office, Rijeka, Croatia	Assoc.Prof. Ines Diminić Lisica, MD, PhD	
General Practice office, Strmec Samoborski, Croatia	Ines Balint, MD	

GOLD 0 and GOLD 1) in the population at risk for COPD and in the general population.

MARKO is a two phase study: Phase I - a cross-sectional; Phase II - prospective, observational, non-interventional cohort study. Flow diagram of subjects through the MARKO study is presented in Fig. 1 and all the assessments during the study are summarized in Table 2.

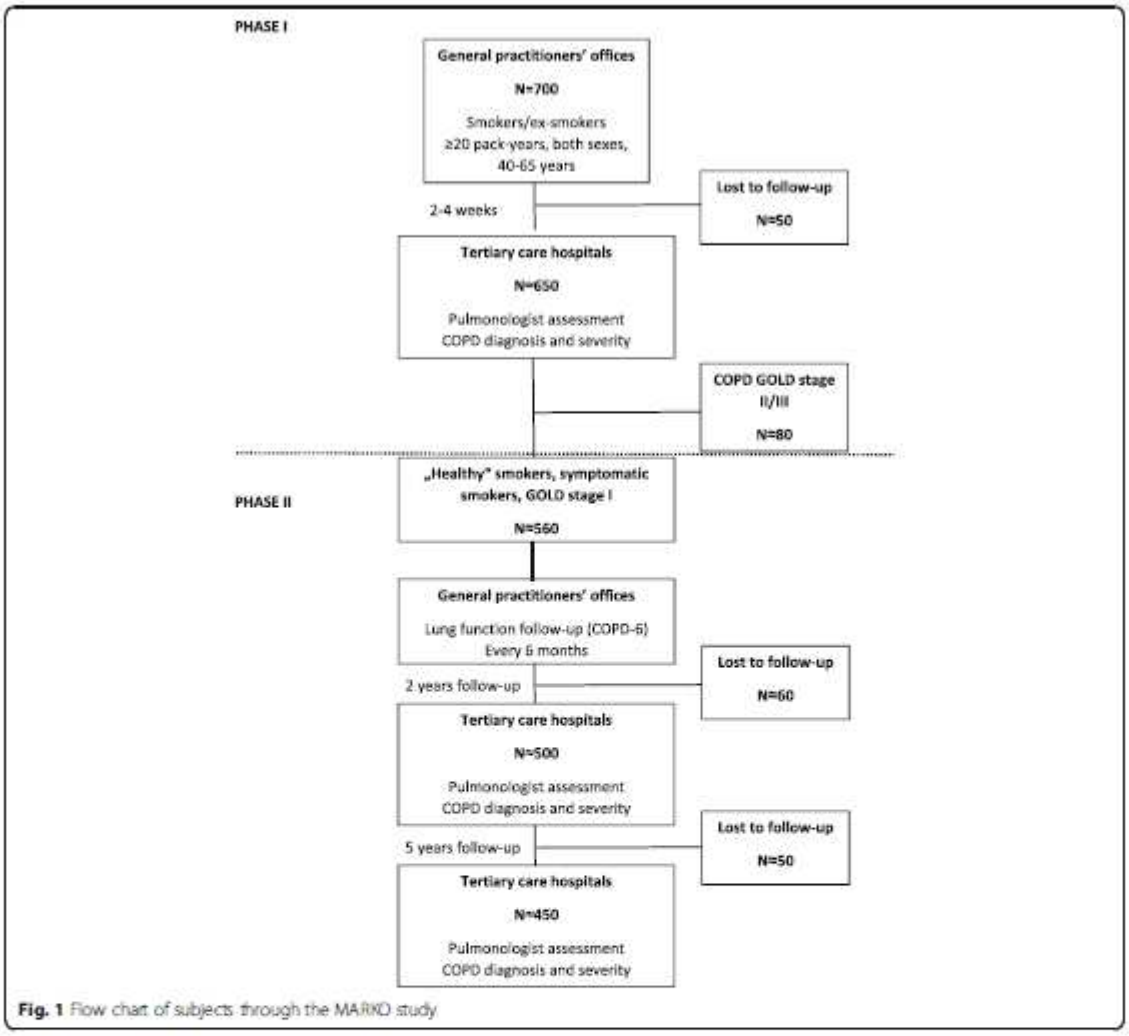

Fig. 1 Flow chart of subjects through the MARKO study

In Phase I up to 700 subjects will be recruited with the risk of COPD by 25 GP's offices (representing 25 GPs) in and around 4 major cities. Patients were approached by their GP during any (unrelated to respiratory problems) visit to the GP's office if they were smokers or ex-smokers of the predefined age group for the study and were prescreened for inclusion/exclusion criteria using a structured interview. Eligible patients were given the Informed consent document with enough time to read it and to discuss any relevant issues regarding the study before consenting. All participants signed written consent for the follow-up study, and separately for biobanking of blood for omics (DNA, RNA, serum and plasma) before taking part in the study and before any procedure was done. Inclusion criteria for the study were defined as: written consent; smokers/ex-smokers; both sexes; aged 40–65 years at inclusion; smoking history of at least 20 pack-years (calculated as a number of cigarettes smoked per day multiplied by the number of years of smoking divided by 20); and no previous diagnosis of COPD. Exclusion criteria were defined as: any clinically relevant chronic disease (cardiovascular, cerebrovascular, diabetes, hepatitis, nephropathy, chronic dialysis, systemic disorder, cancer) significantly affecting HRQoL; ongoing immunosuppressive therapy; preceding acute respiratory disease 4 weeks before inclusion; hospitalization for any reason during the past 3 months; myocardial infarction (MI), cerebrovascular infarction (CVI) or transient ischemic attack (TIA) during the past 6 months; diagnosis of asthma; and an inability to perform the diagnostic protocol. Exclusion criteria were introduced in order not to exclude patients with

Table 2 List of subjects' assessment through different stages of MARKO study

Assessment	Time/place						
	0	2-4 w	6 m	12 m	18 m	24 m	60 m
	GP	P	GP	GP	GP	P	P
Prescreening	X						
Informed consent	X						
Inclusion/exclusion criteria	X						
MARKO questionnaire	X	X				X	X
CAT		X				X	X
SGRQ		X				X	X
History		X				X	X
Physical		X				X	X
EBT before and after cigarette		X					
Lung function							
COPD-6™	X	X	X	X	X		
Spirometry with bronchodilator test		X				X	X
Impulse oscilometry[a]		X					
Lung diffusion capacity		X					
Body plethysmography[a]		X					
Blood sampling (hematology; highly sensitive C-reactive protein; blood stored for DNA, RNA, plasma and serum)[b]		X					
Functional exercise capacity (6-minute walk test)		X					
COPD diagnosis		X				X	X

Legend: w - week, m - month, GP - general practitioner's office, P - pulmonologist at tertiary care hospital, CAT - COPD Assessment Test, SGRQ - St. George Respiratory Questionnaire, EBT - exhaled breath temperature

[a]Impulse oscilometry and body plethysmography was done only in one center

[b]Blood sampling was done only in subjects that signed additional informed consent

comorbidities common in COPD, but to exclude those that represent acute/subacute clinical states/disorders or states of recovery from major clinical disorders representing an absolute or relative contraindication for spirometry or significantly influencing the diagnostic process or an already present diagnosis of respiratory disorder (e.g., asthma). After inclusion patients fill out the self-applicable MARKO questionnaire in the GP's office and measurement of lung function is done using COPD-6™ (4000 COPD-6™ Respiratory Monitor, Vitalograph Ltd., Buckingham, UK) recording the values of forced expiratory volume in 1 s (FEV_1), forced expiratory volume in 6 s (FEV_6 - as a surrogate marker of forced vital capacity [FVC]), FEV_1/FEV_6 (as a surrogate marker of FEV_1/FVC), and lung age. Two to 4 weeks after inclusion eligible patients are referred to one of the 7 tertiary hospital research centers, to a designated team consisting of a pulmonologist, research nurse and lung function laboratory technician where a structured diagnostic workup consisting of the MARKO questionnaire, HRQoL questionnaires (SGRQ and CAT), structured history and physical, exhaled breath temperature (EBT) before (EBTb) and after a smoked cigarette (EBTc), lung function testing with bronchodilator (salbutamol), impulse oscilometry (only in one center), lung diffusion capacity (DL_{CO}), body plethysmography (only in one center), blood sampling (hematology; highly sensitive C-reactive protein [hs-CRP]; blood for DNA, RNA, plasma and serum), functional exercise capacity using 6-min walk test (6MWT), ending with the assessment for diagnosis and severity of COPD according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) and according to the American Thoracic Society/European Respiratory Society (ATS/ERS) recommendation for airway limitation using lower level of normal (LLN) [6, 14].

In Phase II up to 500 subjects will be recruited from Phase I, assessed by designated pulmonologists as 'healthy' smokers, symptomatic smokers (GOLD 0) and as COPD GOLD 1. Selected subjects will be followed for at least 5 years; first assessment after 2 years (±2 months) and the second 5 years (±2 months) after the baseline. During the first 2 years of follow-up all subjects will have regular lung function measurement using COPD-6™ at their GP's office in 6 month intervals. All subjects

will be assessed by the same pulmonologist for diagnosis and severity of COPD according to GOLD and ATS/ERS after 2 and 5 years for the progression of disease defined by three outcome measures: (1) newly diagnosed COPD (ND COPD); (2) disease progression (DP; newly diagnosed COPD + progression to higher severity stage); and (3) high rate of loss of lung function (LoLF; >70 mL/year for post bronchodilator FEV_1). Incidence of newly diagnosed COPD after 2- and 5-years of follow up will be used to identify diagnostic parameters that are most sensitive for early impairment in COPD, to determine the predictability of developed screening MARKO questionnaire alone or with other markers of early impairment in COPD. Blood samples from a subsample of patients who give the consent for omics research will be used to develop omics markers predictive for the future development of COPD and disease progression.

This research will attempt to answer to following questions: (1) can we identify cheap and simple tools that will allow a precise and accurate identification of subjects from a population at risk of developing COPD in the future; (2) is there a combination (pattern) of tools, functional parameters, genetic and biochemical markers that can reliably predict the development of COPD in a population at risk, thus allowing early intervention.

Methods

Screening questionnaire (MARKO questionnaire)

The MARKO questionnaire is a newly constructed HRQoL questionnaire developed by a group of experts; three experienced pulmonologists (ZV, DP, PMAC) and two psychologists (BRV and AKD). The questionnaire comprises 18 questions covering the manifestation and frequency of the symptoms already present at early stages of COPD that could impact the HRQoL in patients. The participants were asked to rate the frequency of their symptoms over a designated period of time (e.g., over the past three months for coughing, shortness of breath, expectoration, and over the past 12 months for pulmonary infections). They also rated their breathing quality and general health status. Furthermore, they reported on shortness of breath during daily life activities requiring different physical strain, and compared their physical abilities and fatigue with respect to their referential age group. The total scores ranged from 0 to 57 points, where the higher scores indicated poorer HRQoL.

Other HRQoL questionnaires

HRQoL will be additionally assessed using 2 standard questionnaires; St. George Respiratory Questionnaire (SGRQ) and COPD Assessment Test (CAT). The SGRQ is a standardized self-administered airways disease-specific questionnaire divided into three subscales: symptoms (8 items), activity (16 items), and impacts (26 items). SGRQ scores were calculated using score calculation algorithms and missing data imputation (if the total number of missing items was ≤10) using the Excel® SGRQ calculator. For each subscale and for the overall questionnaire, scores range from zero (no impairment) to 100 (maximum impairment) [10, 11]. The CAT is a validated, short (8-item) and simple patient completed questionnaire, with good discriminant properties, developed for use in routine clinical practice to measure the health status of patients with COPD. Every item has a scale of 0–5 so the scoring range is from zero (no impairment) to 40 (maximum impairment) [13]. Both HRQoL questionnaires were self-completed by patients before any other procedure was done and after the MARKO questionnaire.

Lung function

Spirometry was performed using computerized pneumotachographs (Jaeger®, CareFusion, CA, USA) using the same procedure at all clinical sites (lung function labs at tertiary hospitals) in agreement with the ATS/ERS standardization [15]. The best of three technically satisfactory efforts was recorded. Bronchodilator test was done with repeated spirometry 20 min after inhalation of 400 mcg of salbutamol using the inhalation chamber. Spirometric parameters (FVC, FEV_1, FEV_1/FVC ratio, peak expiratory flow [PEF], forced expiratory flow between 25 and 75% FVC [FEF_{25-75}]) were recorded as absolute values and as percentage of predicted according to Quanjer [16].

Impulse oscillometry (IOS) was done using Jaeger MasterScreen IOS (Viasys Healthcare, Inc., Yorba Linda, USA) for measurements of respiratory system impedance according to ATS/ERS recommendations and the following IOS data were collected: resistance of the respiratory system at 5 Hz (R_5) and 20 Hz (R_{20}) and reactance at 5 Hz (X_5) [17]. Results were analyzed as absolute values and as percentages of predicted values (%) according to reference equations provided by the manufacturer [18].

Lung volume studies were carried out using a bodyplethysmograph (Ganzhorn, Germany) according to ATS/ERS recommendations [19]. The following parameters were analyzed: airway resistance (RAW), expiratory airway resistance (RAWex), total lung capacity (TLC), residual volume (RV) and the RV/TLC ratio and expressed as percentages of predicted values according to ATS/ERS recommendations [19, 20].

Single-breath diffusing capacity of the lung for carbon monoxide (DL_{CO}) was measured using a rapid carbon monoxide and helium analyzer (Ganzhorn, Germany), which was calibrated prior to each measurement. Values for DL_{CO} and DL_{CO} corrected for alveolar volume (V_A)

[DL_{CO}/V_A] were obtained and are reported as percent predicted values [21].

Exhaled breath temperature

EBT was measured using X-Halo® device (Delmedica Investments, Singapore) according to previously validated method [22]. Patients were requested to inhale freely through the nose and to exhale through the mouth into the device at a rate and depth typical of their normal tidal breathing pattern. The maneuver was continued until the software of the instrument indicated that the measured value was stable, thus fulfilling the criteria of a previously described mathematical model. The tests were carried out at room temperature of 19–25 °C, and at relative humidity of 30–60% in the lung function lab where the atmosphere is controlled and measured. EBT was measured twice on the same occasion (during the initial visit); (1) baseline measurement before any other procedure (lung function, bronchodilator test, 6MWT) at least 1 h after the last smoked cigarette (EBTb) and (2) done only in active smokers 15 min after a smoked cigarette (EBTc) and recorded with precision of 1/100 of a °C. No other procedure apart from cigarette smoking was carried out between the two EBT measurements.

Blood sampling and storage

Before initializing the sample collection process, it was important to ensure the correct order of blood collection. The PAXgene blood RNA tube had to be the last tube drawn in the study procedure process. Before sample collection, all tubes were labeled with patient identification number. Blood samples for serum were drawn at minimal volume of 3 ml in serum separation vacuum tubes (containing Z Serum Clot Activator gel) and sent to the laboratory. Samples were kept at room temperature for at least 30 min, but had to be processed within 2 h of blood collection. Upon centrifugation at 3000 rpm for 10 min, a minimum of 300 μl of serum was separated for further detection of hs-CRP level. The rest of the serum sample from every subject was transferred to a cryotube vial and stored at −20 °C.

Blood samples for plasma were drawn at minimal volume of 3 ml in K2EDTA vacuum blood collection tubes and were inverted several times to ensure proper mixing of additive with blood. Samples were sent to the laboratory where 200 μl of blood was separated for the purpose of complete blood cell hematological analysis. The remaining blood volume was kept at room temperature for at least 30 min, but had to be processed within 2 h of blood collection. Samples were then centrifuged at 3500 rpm for 10 min. The plasma sample from every subject was transferred to a cryotube vial and stored at −20 °C.

Blood samples for DNA extraction were drawn at minimal volume of 2 ml in K2EDTA vacuum blood collection tubes and were inverted several times to ensure proper mixing of the additive with blood. Samples were sent to the laboratory where they were kept at room temperature for 30 min, and inverted again before their transfer to a cryotube vial which was stored at −20 °C.

Blood samples for RNA extraction were drawn at volume of 2.5 ml in PAXgene blood RNA tubes, which were kept at room temperature prior to use. After blood collection, the PAXgene blood RNA tubes were immediately gently inverted 8–10 times to ensure proper mixing of additive with blood, after which they were stored at −20 °C.

Laboratory analyses were done in local laboratories and included complete blood count, white blood cells differential count, hematocrit, hemoglobin and hs-CRP.

Functional exercise capacity was assessed using 6MWT according to the ATS guidelines and expressed as walked distance in meters and as % of predicted according to Trooster et al. [23, 24].

Data analyses

Data analyses are envisaged using STATISTICA version 12 (StatSoft, Inc., OK, USA), MedCalc Statistical Software version 16.8.4 (MedCalc Software bvba, Ostend, Belgium; https://www.medcalc.org; 2015) and RUMM2030 (RUMM Laboratory, Perth, Australia). Sample size calculation was done based on the following assumptions: we expected to find 25% patients in different stages of COPD according to the airflow limitation. We therefore expected that the sample would consist of 75% of 'healthy' and symptomatic smokers, 12.5% patients with COPD GOLD 1, 6.25% in GOLD 2 and 6.25% in GOLD 3 or 4 stages at initial visit with the expected difference in the MARKO questionnaire scores of 2 points and SD od 2.5 points between 'healthy' smokers vs. symptomatic smokers vs. COPD GOLD 1/2 having a statistical power of >80% with alpha = 0.05 for the sample size of at least 500 subjects. The expected yearly incidence rate for GOLD 1 and progression from GOLD 1 to GOLD 2 is planned at 10/per 100 patient-years.

Phase I data analysis will primarily focus on primary validation of the MARKO questionnaire (its psychometric characteristics), convergent and discriminant validity comparing data between different subgroups and with other HRQoL questionnaires (CAT and SGRQ). Categorical data will be compared between subgroups using chi-square test or Fisher exact test and continuous variables using Mann-Whitney *U*-test and Kruskal-Wallis ANOVA (non-normal distribution expected). Metric characteristics of the MARKO questionnaire will also be analyzed using Cronbach's alpha, Lin's concordance and Spearman's correlation coefficients, analyzing inner consistency,

test-retest reliability, and association with other measures of HRQoL and health status.

Phase II data analysis will focus on secondary validation of the MARKO questionnaire to determine the construct validity and predictability (alone or in combination with other markers) for future development and/or progression of COPD. Construct validity of the MARKO questionnaire will be assessed using factorial analysis to confirm the number of factors the questionnaire is measuring with calculations of inter- and intracorrelations between the factors and items. Primary objective will be to create a questionnaire with the smallest number of items with reliable predictive properties. Identifying items for potential deletion will be based on a hierarchical process: age and sex bias, floor and ceiling effects, item to total correlation and tests of redundancy (inter-item correlation). Rasch analysis will be used to identify items with the best fit to a predictive model. Utility of different markers for disease progression will be assessed using generalized linear/nonlinear regression models and expressed as odds ratio (OR) with 95% confidence intervals (CIs). Predictive power for the models will be presented using receiver operator curve (ROC) analysis with AUC (with 95% CIs) together with associated criterion, sensitivity, specificity and positive (PPV) and negative predictive (NPV) values. $P < 0.05$ will be used as statistically significant for all analyses with correction for multiple comparisons.

Discussion

This is one of the first cohort studies attempting to establish the predictors and incidence of COPD in presymptomatic stage before clinical diagnosis and clinically documented end organ damage. Having in mind the progressive nature of COPD with high morbidity and early mortality, our aim is to try to develop and identify simple tools and markers or a pattern of the afore mentioned in order to allow diagnosis as early as possible, before a significant fall in lung function. The problem when using lung function for diagnosing COPD lies in the significant variability of what represents normal (reference) values and the need for a significant expertise in providing technically appropriate measurement, thus limiting its use for screening purposes or making an early diagnosis [13, 14, 25]. Numerous studies have been focused on testing simple screening tools (questionnaires and/or simplified flow measurement devices) for already developed COPD, in order to surpass the limitations of spirometry obtaining a specificity of up to 84.4%, with inadequate blinding between an index test and spirometry being a major source of bias in these studies [26]. We have managed to surpass this major bias using the subsample from Phase I of the MARKO study and showing that lung function testing with COPD-6™ can substitute spirometry testing in cases where it is not readily available to the patient/physician, but bearing in mind that the traditional cutoff value of <0.7 for FEV_1/FEV_6 ratio, cannot be the only criterion for COPD diagnosis and/or further referral [27]. Early diagnosis and early intervention (based on data from pathophysiological studies) is highly advocated and studies already starting chronic bronchodilator treatment in early stages of COPD (in GOLD stages 1 and 2) have been initiated [7, 28–30]. In order for it to be possible to intervene even earlier in the course of disease, with intervention being specific (not harmful), it is very important for method(s) to be developed to be highly specific in identifying subjects that will develop COPD. Genetic testing in studies like UK BiLEVE, using large population samples, showed that contrary to previous genetic studies significant genetic signals predictive for COPD irrespective of smoking can be picked up, identifying specific mechanisms underlying airflow limitation [8]. However these data should be further confirmed using other populations and follow-up.

Phase I of the MARKO study will be used to test the psychometric characteristics of the MARKO questionnaire; to evaluate discriminative power of the MARKO questionnaire together with COPD-6™ measurements between different developmental stages of COPD; to identify diagnostic parameters that are most sensitive for early impairment in COPD (discriminate between COPD GOLD 0 and 1); to compare the MARKO questionnaire with other measures used to evaluate patients (HRQoL, history, physical, lung function, functional capacity); and to assess the prevalence of different stages of COPD (specifically GOLD 0 and 1) in the population at risk for COPD. Preliminary data from Phase I of initial validation of the MARKO questionnaire showed the potential for the MARKO questionnaire to assess early health status changes in smokers at risk for chronic obstructive pulmonary disease [31]. Although exclusion criteria were introduced to exclude subjects with acute/subacute clinical disorders or states of recovery from major clinical disorders representing an absolute or relative contraindication for spirometry or significantly influencing the diagnostic process, the data analysis showed that more than half (56.3%) of the recruited subjects had one or more (up to 3) comorbidities (28.6% hypertension, 10% dyslipidemia, 5.2% diabetes and 4.9% peptic syndrome, etc.) [31].

Phase II of the MARKO study will be used to test the primary endpoint of the study: to validate the MARKO questionnaire as a tool to be used alone or combined with other markers in identifying subjects at risk (smokers/ex-smokers with relevant exposure to tobacco smoke) who will develop COPD during the follow-up. Based on previously published data that EBT could be a sensitive marker of airways inflammation [21] we introduced it as a possible predictive marker for future COPD. Recently published

data from Carpagnano GE et al., and from our group (preliminary data from the MARKO study) showed the sensitivity of EBT to cigarette smoke and the potential to predict future development of COPD in current smokers [32, 33]. All other parameters were measured/gathered at the start of the study for a detailed phenotype/endotype of recruited subjects and as possible predictive markers for future COPD.

We anticipate that the MARKO study will give us answers regarding the predictability and discriminative power of a newly developed HRQoL tool (MARKO questionnaire) alone; or in combination with other markers, such as EBT (using new protocol of assessment) [33], lung function, 6MWT, genomics, transcriptomics, proteomics. We believe that the results of this study will improve our understanding of the development of COPD, identify some underlying pathophysiological pathways, and offer to sensitive smokers/ex-smokers the possibility of an earlier intervention, thus improving the management of COPD.

Abbreviations

6MWT: 6-min walk test; ANOVA: Analysis of variance; ATS: American Thoracic Society; AUC: Area under the curve; CAT: COPD Assessment Test; CCQ: Clinical COPD Questionnaire; CI: Confidence interval; COPD: Chronic obstructive pulmonary disease; CRP: C reactive protein; CRQ: Chronic Respiratory Questionnaire; CVI: Cerebrovascular infarction; DL_{CO}: Diffusing capacity of the lung for carbon monoxide; DP: Disease progression; EBT: Exhaled breath temperature; EBTb: Baseline EBT; EBTc: EBT after smoked cigarette; ERS: European Respiratory Society; FEF_{25-75}: Forced expiratory flow between 25 and 75% FVC; FEV_1: Forced expiratory volume in 1. second; FVC: Forced vital capacity; GCP: Good Clinical Practice; GOLD: Global initiative for chronic Obstructive Lung Disease; GP: General practitioner; HRQoL: Health Related Quality of Life; hs: High-sensitivity; IOS: Impulse Oscillometry; LoLF: Rate of loss of lung function; MI: Myocardial infarction; ND: Newly diagnosed; NPV: Negative predictive value; OR: Odds ratio; PEF: Peak expiratory flow; PPV: Positive predictive value; ROC: Receiver operator curve; SGRQ: Saint George Respiratory Questionnaire; TIA: Transient ischemic attack; WBC: White blood cell count

Acknowledgements

The authors wish to thank the consultant Prof. Peter MA Calverley for his valuable advice regarding study design and the construction/design and review of the MARKO questionnaire together with psychologists Professors Adrijana Kolčec, Duknić and Biserka Radošević-Vidaček. The authors would also like to thank the following researchers who helped in the recruitment of participants and the data collection: Vjekoslava Ameršek, MD; Ines Balint, MD; Mesim Bezdrov, MD; Mirjana Bezdrov, MD; Ivana Boban, MD; Ljiljana Bulat Kardum, MD, PhD; Ines Dominić Lisica, MD, PhD; Albina Durnić, MD, PhD; Ljiljana Ismić, MD; Tajana Jalušić Glunčić, MD; Renata Grgurić, MD; Monika Jeđud, MD; Đivo Ljubičić, MD; Tina Lukić, MD; Ljiljana Lulić-Karapetrić, MD; Suzana Maltar-Delija, MD; Dubravka Margaretić, MD; Davorka Martinković, MD; Zdenka Meštrović, MD; Nataša Mrduljaš-Đujić, MD; Jasna Nagyszombaty-Šarić, MD; Darja Nelken-Bestvina, MD; Sanja Popović Grle, MD, PhD; Sanda Pribić, MD; Jadranka Radman, MD; Rosanda Rosandić Plasevoli, MD; Alen Stojanović, MD; Karla Tudja, MD; Neven Tudorić, MD, PhD; Bojana Vukanjac, MD; Vanja Vuli, MD.

Funding

The study was in part funded by an unrestricted grant from GlaxoSmithKline (GSK eTrack number CRT114338). Prof. Davor Plavec and Dr. Žarko Vrbica as principal investigators and the Children's Hospital Srebrnjak have for the purpose of investigator initiated study MARKO (ClinicalTrials.gov Identifier NCT01550679) received an unrestricted grant from GlaxoSmithKline. GlaxoSmithKline has not influenced the study design or study protocol, has no ownership rights over data gathered by the study and has no influence on this and further publications arising from data gathered through this study.

Availability of data and material

As this is a clinical dataset, data is not publicly available because it would breach the ethics committee approval (issued in 2010) and informed consent signed by the participants of this trial (signed in 2010–2012).

Authors' contributions

Authorship of ŽV and MI. should be evaluated as equally contributing first authors. MI. participated in the study design and coordination, data acquisition, and drafting and revision of the manuscript for important intellectual content. ŽV participated in conceiving the study, its design and coordination and drafting and revision of the manuscript for important intellectual content. IG participated in study design and coordination, data acquisition and drafting and revision of the manuscript for important intellectual content. SI. participated in study coordination, data acquisition and drafting and revision of the manuscript for important intellectual content. IJ participated in study coordination, data acquisition and drafting and revision of the manuscript for important intellectual content. DP participated in conceiving the study, its design and coordination, data acquisition, statistical analysis and drafting and revision of the manuscript for important intellectual content. All authors contributed to the original ideas and drafting or revision of the manuscript for important intellectual content and gave final approval of the final version of the manuscript.

Competing interests

Prof. Davor Plavec and Dr. Žarko Vrbica as principal investigators and the Children's Hospital Srebrnjak have for the purpose of investigator initiated study MARKO (ClinicalTrials.gov Identifier NCT01550679) received an unrestricted grant from GlaxoSmithKline (GSK eTrack number: CRT114338) based on the study protocol application. GlaxoSmithKline has not influenced the study design or study protocol, has no ownership rights over data gathered by the study and has no influence on this and further publications arising from data gathered through this study. Authors declare that they have no other competing interests regarding this study.

Consent for publication

Not applicable.

Ethics approval and consent to participate

The study was approved by the Children's Hospital Srebrnjak Ethics Committee (No. 01–2191/1–2010) and conducted according to the Declaration of Helsinki and other relevant international and national laws. The patients were approached by their GPs during any (unrelated to respiratory problems) visit to their office if they were smokers or ex-smokers of the predefined age group for the study together with the prescreening for inclusion/exclusion criteria using a structured interview. Eligible patients were given the Informed consent document with enough time to read it and to discuss any relevant issues regarding the study before they signed the written consent. They were informed about the prospective nature of the study and their right to withdraw their consent and claim the withdrawal of all gathered data and destruction of all biological samples at any time without any explanation, obligation or consequence on their part. All participants signed the written consent before starting any procedure for the study.

Author details

[1]Department of Pulmonology and Immunology, General Hospital Dubrovnik, Dr. Roka Mišetića 2, Dubrovnik, Croatia. [2]University of Dubrovnik, Branitelja Dubrovnika 29, Dubrovnik, Croatia. [3]Department of Pulmonology, University Hospital Center Osijek, Josipa Huttlera 4, Osijek, Croatia. [4]Faculty of Medicine, J.J. Strossmayer University of Osijek, Ulica cara Hadrijana 10E, Osijek, Croatia. [5]Department of Pulmonology, University Hospital Center Split, Spinčićeva 1, Split, Croatia. [6]Department of Internal Medicine, University Hospital Center Osijek, Josipa Huttlera 4, Osijek, Croatia. [7]Research Department, Children's Hospital Srebrnjak, Srebrnjak 100, Zagreb, Croatia.

Received: 17 March 2016 Accepted: 1 February 2017
Published online: 10 February 2017

References

1. Kim W, Ling SH, Coxson HO, English JC, Yee J, Levy RD, et al. The association between small airway obstruction and emphysema phenotypes in COPD. Chest. 2007;131:1372–8.
2. Miravitlles M, Anzueto A. Insights into interventions in managing COPD patients: lessons from the TORCH and UPLIFT® studies. Int J Chron Obstruct Pulmon Dis. 2009;4:185–201.
3. Greulich T, Koczulla R, Vogelmeier C, Bals R. Chronic obstructive pulmonary disease (COPD) as a systemic disease. Dtsch Med Wochenschr. 2009;134:1231–5.
4. Jenkins CR, Jones PW, Calverley PM, Celli B, Anderson JA, Ferguson GT, et al. Efficacy of salmeterol/fluticasone propionate by GOLD stage of chronic obstructive pulmonary disease: analysis from the randomised, placebo-controlled TORCH study. Respir Res. 2009;10:59.
5. Antoniu SA. Descriptors of dyspnea in obstructive lung diseases. Multidiscip Respir Med. 2010;30:216–9.
6. Global Initiative for Chronic Obstructive Lung Disease (GOLD). 2015. Available from: http://goldcopd.org/. Accessed 15 May 2015.
7. Nardini S, Annesi-Maesano I, Del Donno M, Delucchi M, Bettoncelli G, Lamberti V, et al. The AIMAR recommendations for early diagnosis of chronic obstructive respiratory disease based on WHO/GARD model. Multidiscip Respir Med. 2014;9:46.
8. Wain LV, Shrine N, Miller S, Jackson VE, Ntalla I, Soler Artigas M, et al. Novel insights into the genetics of smoking behaviour, lung function, and chronic obstructive pulmonary disease (UK BiLEVE): a genetic association study in UK Biobank. Lancet Respir Med. 2015;3:769–81.
9. Guyatt GH, Berman LB, Townsend M, Pugsley SO, Chambers LW. A measure of quality of life for clinical trials in chronic lung disease. Thorax. 1987;42:773–8.
10. Jones PW, Quirk FH, Baveystock CM. The St George's Respiratory Questionnaire. Respir Med. 1991;85(Suppl B):25–31):33–7.
11. Jones PW, Quirk FH, Baveystock CM, Littlejohns P. A self-complete measure for chronic airflow limitation – the St George's Respiratory Questionnaire. Am Rev Respir Dis. 1992;145:1321–7.
12. van der Molen T, Willemse BW, Schokker S, ten Hacken NH, Postma DS, Juniper EF. Development, validity and responsiveness of the Clinical COPD Questionnaire. Health Qual Life Outcomes. 2003;1:13.
13. Jones PW, Harding G, Berry P, Wiklund I, Chen WH, Kline LN. Development and first validation of the COPD Assessment Test. Eur Respir J. 2009;34:648–54.
14. Quanjer PH, Stanojevic S, Cole TJ, Baur X, Hall GL, Culver BH, et al. and the ERS Global Lung Function Initiative. Multi-ethnic reference values for spirometry for the 3–95 years age range: the Global Lung Function 2012 equations. Eur Respir J. 2012;40:1324–43.
15. Oostveen E, MacLeod D, Lorino H, et al. The forced oscillation technique in clinical practice: methodology, recommendations and future developments. Eur Respir J. 2003;22:1026–41.
16. Vogel J, Smidt U. Impulse Oscillometry. Analysis of lung mechanics in general practice and the clinic, epidemiological and experimental research. Frankfurt am Main: pmi Verlagsgruppe GmbH. 1994.
17. Wanger J, Clausen JL, Coates A, et al. Standardisation of the measurement of lung volumes. Eur Respir J. 2005;26:511–22.
18. Stocks J, Godfrey S, Beardsmore C, et al. Plethysmografic measurements of lung volume and airway resistance. Eur Respir J. 2001;17:302–12.
19. Roca J, Rodriguez-Roisin R, Cobo E, et al. Single-breath carbon monoxide diffusing capacity prediction equations from a Mediterranean population. Am Rev Respir Dis. 1990;141:1026–32.
20. Popov TA, Dunev S, Kralimarkova TZ, Kraeva S, Dudbske LM. Evaluation of a simple, potentially individual device for exhaled breath temperature measurement. Respir Med. 2007;101:2044–50.
21. ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories. ATS statement guidelines for the six-minute walk test. Am J Respir Crit Care Med. 2002;166:111–7.
22. Troosters T, Gosselink R, Decramer M. Six minute walk distance in healthy elderly subjects. Eur Respir J. 1999;14:270–4.
23. Quanjer PH, Brazzale DJ, Boros PW, Pretto JJ. Implications of adopting the Global Lungs Initiative 2012 all-age reference equations for spirometry. Eur Respir J. 2013;42:1046–54.
24. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. ATS/ERS Task Force: ATS/ERS Task Force. Standardisation of spirometry. Eur Respir J. 2005;26:319–38.
25. Lam DC, Hui CK, Ip MS. Issues in pulmonary function testing for the screening and diagnosis of chronic obstructive pulmonary disease. Curr Opin Pulm Med. 2012;18:104–11.
26. Haroon S, Jordan R, Takwoingi Y, Adab P. Diagnostic accuracy of screening tests for COPD: a systematic review and meta-analysis. BMJ Open. 2015;5:e008133.
27. Labor M, Vrbica Ž, Gudelj I, Labor S, Plavec D. Diagnostic accuracy of a pocket screening spirometer in diagnosing chronic obstructive pulmonary disease in general practice: a cross sectional validation study using tertiary care as a reference. BMC Fam Pract. 2016;17:112.
28. Csikesz NG, Gartman EJ. New developments in the assessment of COPD: early diagnosis is key. Int J Chron Obstruct Pulmon Dis. 2014;9:277–86.
29. Elbehairy AF, Webb KA, Neder JA, O'Donnell DE. Should mild COPD be treated? Evidence for early pharmacological intervention. Drugs. 2013;73:1991–2001.
30. Li X, Zhou Y, Chen S, Zheng J, Zhong N, Ran P. Early intervention with tiotropium in Chinese patients with GOLD stages I-II chronic obstructive pulmonary disease (Tie-COPD): study protocol for a multicentre, double-blinded, randomised, controlled trial. BMJ Open. 2014;4:e003991.
31. Vrbica Ž, Labor M, Kolčec Đukrić A, Radolević-Vidaček B, Gudelj I, Labor S, Jurić I, Calverley PM, Plavec D. Development and the initial validation of a new self-administered questionnaire for an early detection of health status changes in smokers at risk for chronic obstructive pulmonary disease (MARKO questionnaire). Croat Med J. 2016;57:425–33.
32. Carpagnano GE, Ruggieri C, Sciascia G, Storto MM, Zoppo L, Foschino-Barbaro MP. Is the Exhaled Breath Temperature Sensitive to Cigarette Smoking? COPD. 2016;13:642–6.
33. Labor M, Vrbica Ž, Gudelj I, Labor S, Jurić I, Plavec D. Exhaled Breath Temperature as a Novel Marker of Future Development of COPD: Results of a Follow-Up Study in Smokers. COPD. 2016;13:741–9.

3.2. Development and the initial validation of a new self-administered questionnaire for an early detection of health status changes in smokers at risk for chronic obstructive pulmonary disease (MARKO questionnaire).

Croat Med J. 2016;57:425-33
https://doi.org/10.3325/cmj.2016.57.425

Development and the initial validation of a new self-administered questionnaire for an early detection of health status changes in smokers at risk for chronic obstructive pulmonary disease (MARKO questionnaire)

Žarko Vrbica[1,2*], Marina Labor[3,4*], Adrijana Košćec Đuknić[5], Biserka Radošević-Vidaček[6], Ivan Gudelj[7], Slavica Labor[3,4], Iva Jurić[8], Peter MA Calverley[9], Davor Plavec[10,4]; for the MARKO study group

[1]Department of Pulmonology and Immunology, General Hospital Dubrovnik, Dubrovnik, Croatia

[2]University of Dubrovnik, Dubrovnik, Croatia

[3]Department of Pulmonology, University Hospital Center Osijek, Osijek, Croatia

[4]Faculty of Medicine, J.J. Strossmayer University of Osijek, Osijek, Croatia

[5]University of Zagreb Centre for Croatian Studies, Borongaj Campus, Zagreb, Croatia

[6]Institute for Medical Research and Occupational Health, Zagreb, Croatia

[7]Department of Pulmonology, University Hospital Center Split, Split, Croatia

[8]Department of Internal Medicine, University Hospital Center Osijek, Osijek, Croatia

[9]University of Liverpool, Liverpool, UK

[10]Research Department, Children's Hospital Srebrnjak, Zagreb, Croatia

*The first two authors contributed equally.

Aim To develop and do an initial validation of a new simple tool (self-administered questionnaire) that would be sensitive and specific enough to detect early changes in smokers leading to future development of chronic obstructive pulmonary disease (COPD).

Methods 224 consecutive participants (50.9% women), with mean ± standard deviation age of 52.3 ± 6.7 years, 37.5 ± 16.7 pack-years smoking history (85.8% active smokers), and no prior diagnosis of COPD were recruited. The MARKO questionnaire was self-administered twice; at the general practitioner's office and after 2-4 weeks at the tertiary care hospital. Participants were assessed for COPD by a pulmonologist after filling in a quality of life (QoL) questionnaires, history-taking, physical examination, lung function test, 6-minute walk test, and laboratory tests. They were divided into four subgroups: "healthy" smokers, symptomatic smokers, and smokers with mild and moderately severe COPD.

Results Psychometric analyses indicated that the 18-item questionnaire had a very good internal consistency (Cronbach's alpha = 0.91) and test-retest reliability for a four week period (pc = 0.89, 95% confidence interval [CI] 0.85-0.92, Lin's concordance). A significant correlations of MARKO scores were found with two QoL questionnaires; r = 0.69 ($P < 0.001$) and r = 0.81 ($P < 0.001$). Receiver operating characteristic curve analysis showed an area under the curve of 0.753 (95% CI 0.691-0.808, $P < 0.001$), with a sensitivity of 71.83% and specificity of 64.24% to discriminate "healthy" smokers from other subgroups.

Conclusion Based on psychometric analyses and high convergent validity correlation with already validated QoL questionnaires, the newly developed MARKO questionnaire was shown to be a reliable self-administered short health status assessment tool.

Trial registration: Clinicaltrial.gov NCT01550679

Received: March 21, 2016
Accepted: October 24, 2016

Correspondence to:
Davor Plavec
Head of Research Department
Children's Hospital Srebrnjak
Srebrnjak 100
10000 Zagreb, Croatia
plavec@bolnica-srebrnjak.hr

Chronic obstructive pulmonary disease (COPD) is one of the major causes of chronic morbidity and mortality throughout the world (1). Millions of people suffer from this disease for years, and die prematurely from it or its complications, thus producing a significant impact on the health care system and economy. Since COPD is a preventable and treatable disease, early detection is very important (1,2). Chronic airflow limitation, which is a major characteristic of COPD, is caused by a mixture of small airways lesions and parenchyma destruction caused by chronic inflammation. According to the Global Initiative for Chronic Obstructive Lung Disease (GOLD), to establish the diagnosis, the patient has to have a significant exposure, characteristic symptoms, and a significant degree of airflow limitation (Tiffeneau index <0.7) (1). American Thoracic Society/European Respiratory Society (ATS/ERS) use even more stringent criteria for a significant airflow limitation based on the lower limit of normal (LLN), arguing that using a single point criteria for all age groups significantly under- or overestimates the incidence and prevalence of COPD in different age groups (3). On the other hand, pathophysiological changes, symptoms, and diminished health related quality of life (HRQoL) often precede clinically significant airflow limitation (1). Even though cigarette smoking is the major cause of COPD, only a fraction (<1/3) of the smokers develop the disease. Based on the recently published data from a subsample (n = 50 008) of the UK Biobank, there are significant shared genetic mechanisms underlying airway limitation, COPD, and smoking addiction (4). Despite recommendations for an early diagnosis (1,2), up until now, there have been no predictive parameters to evaluate the risk for developing COPD in a particular person exposed to tobacco smoke. Interdisciplinary Association for Research in Lung Disease (Associazione Scientifica Interdisciplinare per lo Studio delle Malattie Respiratorie, AIMAR) guidelines recommend using a stepwise approach that starts with the screening questionnaire as a first step in the identification of a high risk population. Already validated HRQoL questionnaires like St' George Respiratory Questionnaire (SGRQ) or COPD Assessment Test (CAT) have not been developed and validated for such a purpose (5,6). The aim of our study was to construct, develop, and conduct an initial validation of a new simple tool (self-administered questionnaire) that would be sensitive and specific enough to detect early changes in smokers leading to future development of COPD.

METHODS

This study was a part of broader research project "Early Detection of COPD Patients in GOLD 0 (Smokers) Population – MARKO Project." The details of the protocol of the MARKO project can be found at https://clinicaltrials.gov/ct2/show/NCT01550679. The study was approved by the Children's Hospital Srebrnjak Ethics Committee and conducted according to the most recent version of the Declaration of Helsinki, Good Clinical Practice, and other relevant international and national laws. All participants signed the Informed consent before starting any procedure related to the study.

Pilot study for understanding/comprehension

A cross-sectional study was conducted in 2009 on a primary care level in a large city (Zagreb and surroundings) to assess the prevalence of COPD. A subgroup of 138 patients of both sexes was chosen based on a previously diagnosed COPD for this pilot study. The study was undertaken through 17 general practitioners' (GP) offices that possessed the COPD-6™ pocket screening spirometer (4000 COPD-6™ Respiratory Monitor, Vitalograph Ltd, Buckingham, UK).

The GPs were asked to collect the data on all the patients who were active smokers with ≥20 pack-years smoking history and over 40 years of age, irrespective of the reason of their visit, about their chronic respiratory conditions including COPD, asthma, or any other respiratory condition, respiratory therapy, and exacerbations during the past year. They were also asked to measure lung function using the COPD-6™ (forced expiratory volume in 1 second [FEV_1], forced expiratory volume in 6 seconds [FEV_6] as a surrogate measure for forced vital capacity [FVC], FEV_1/FEV_6 ratio, and lung age). Patients (n = 138) with COPD assessed the readability and comprehension of the MARKO questionnaire items. Each of the 18 items in MARKO questionnaire was rated on a scale from 1-4 (1 meaning lowest level of understanding/comprehension and 4 meaning the complete understanding/comprehension of each item) (Supplementary material).

Validation study

Participants. The study conducted between 2010-2013 included 224 consecutive participants (50.9% women) with mean ± standard deviation age of 52.3 ± 6.7 years and 37.5 ± 16.7 pack-years smoking history (85.8% active smokers). They were recruited at 15 GP offices representing an equal number of GPs in two major cities (Zagreb and surroundings 7 GPs, Split and surroundings 8 GPs). The participants were approached by their GPs during a random visit to their office (not related to respiratory problems) if they were smokers or ex-smokers of the predefined age group.

The pre-screening for inclusion/exclusion criteria was conducted through a structured interview. The inclusion criteria were that participants have to have signed the written consent; be smokers/ex-smokers of either sex aged 40-65 years with a smoking history of at least 20 pack-years (calculated as the number of cigarettes smoked per day multiplied by the number of years of smoking divided by 20); and have no previous diagnosis of COPD. The exclusion criteria were any clinically relevant chronic disease (cardiovascular, cerebrovascular, diabetes, hepatitis, nephropathy, chronic dialysis, systemic disorder, cancer) significantly affecting QoL at the time of the first visit; immunosuppressive therapy; preceding acute respiratory disease four weeks before the visit; hospitalization for any reason during past three months; myocardial infarction, cerebrovascular infarction or transient ischemic attack during past six months; diagnosis of asthma; and an inability to perform the diagnostic protocol. After the diagnostic workup participants were divided into four subgroups defined as "healthy" smokers (no respiratory symptoms and $FEV_1/FVC \geq 0.7$, n = 72), symptomatic smokers (chronic respiratory symptoms as dyspnea, cough and/or sputum production and $FEV_1/FVC \geq 0.7$, n = 110), COPD GOLD 1 (chronic respiratory symptoms, $FEV_1/FVC < 0.7$ and $FEV_1 \geq 80\%$ predicted, n = 23), and COPD GOLD 2 (chronic respiratory symptoms, $FEV_1/FVC < 0.7$ and $FEV_1 < 80\%$ and $\geq 50\%$ predicted, n = 19) (1).

Measuring instruments

The MARKO questionnaire was constructed and developed in the Croatian language for the purpose of this study by a group of experts: three medical doctors (pulmonologists ŽV, DP, and PMAC) and two psychologists (BRV, AKE). It was constructed in the Croatian language because the whole MARKO study was planned and performed in Croatia and did not involve participants from other countries. The questionnaire comprised 18 questions covering the manifestation and frequency of the symptoms present at the early stages of the COPD that could impact the patients' HRQoL. The participants were asked to rate the frequency of their symptoms over a designated period of time (eg, over past three months for coughing, shortness of breath, expectoration, and over past 12 months for pulmonary infections). They also rated their breathing quality and general health status. Furthermore, they reported on the shortness of breath during daily life activities requiring different physical strain, and compared their physical abilities and fatigue with respect to their referential age group. The total scores ranged from 0 to 57 points, with the higher scores indicating poorer HRQoL.

CAT is a validated, short (8-item), and simple self-administered questionnaire, with good discriminant properties, developed for use in routine clinical practice to measure the health status of patients with COPD (6). The test was developed using Rasch analysis as a single dimensional construct. Internal consistency was excellent with Cronbach's $\alpha = 0.88$ and a good test-retest reliability (intraclass correlation coefficient = 0.8). Every item is rated on a six point scale from 0 to 5. Total scores range from 0 (indicating no impairment) to 40 (indicating maximum impairment). It is openly accessible and available in more than 60 languages. It was validated in 6 different countries using 4 different languages and translated to the Croatian language using an internationally recommended procedure (7).

SGRQ was designed to measure the overall health status and well-being of the patients with obstructive airways disease (5). It is a standardized self-administered airways disease-specific questionnaire divided into three domains: symptoms (8 items), activity (16 items), and impacts (26 items). Internal consistency (Cronbach's α) for these domains for COPD was 0.61, 0.90, and 0.88, respectively. For each domain and for the overall questionnaire, scores range from zero (no impairment) to 100 (maximum impairment). The questionnaire is available in more than 70 languages and openly accessible. SGRQ was not previously validated for the Croatian language but was translated using an international recommended procedure and used widely in many COPD clinical trials in Croatia (7). The SGRQ scores in our study were calculated using score calculation algorithms and missing data imputation (if total number of missing items was ≤10) using the Excel® SGRQ calculator.

Procedure

The purpose of this initial validation was to understand the basic psychometric characteristics of this newly constructed questionnaire and determine how it compares to the already existing and validated HRQoL questionnaires used for COPD, like CAT and SGRQ. Also it was important to understand if the newly developed questionnaire discriminates between all 4 subgroups of participants. We also understand that we have different domains and some redundant questions as they differ in the level of symptoms severity. They were put into the construct on purpose because the final evaluation would be made based on the results of participants' follow-up. The main purpose why this questionnaire was developed was to try to pick-up early changes in HRQoL that are predictive for the future develop-

ment of COPD in smokers at risk, or with a progression of an early COPD. As there are no up-to-date instruments that can be compared with this, the second validation will be done using follow-up data that will allow us to discard redundant questions and fully analyze the construct validity of MARKO questionnaire. The MARKO questionnaire was self-administered twice in a validation study; first at the GP's office and after 2-4 weeks at the tertiary care hospital during pulmonologist's assessment. During the pulmonologist's assessment the staff and the participant were blinded for the results of MARKO questionnaire obtained at the GP's office. At the tertiary care hospital participants were referred to a designated team consisting of a pulmonologist, study nurse, and lung function laboratory technician. They filled in the self-administered MARKO questionnaire followed by CAT and SGRQ, after which they went through a structured and predefined diagnostic workup (history-taking, physical examination, lung function with bronchodilator test, 6-minute walk test, laboratory tests) to determine the diagnosis and staging of their COPD according to the GOLD and were divided in four subgroups that were used for further comparisons as previously described (1).

Data analyses

Data analyses were conducted using STATISTICA version 12 (StatSoft, Inc., OK, USA) and MedCalc Statistical Software version 15.8 (MedCalc Software bvba, Ostend, Belgium). Categorical data are presented as absolute numbers and percentages. Quantitative data are expressed as mean and standard deviation (SD) or median and interquartile range (IQR). Normality of distribution was assessed using Kolmogorov-Smirnov test. Metric characteristics of the MARKO questionnaire were analyzed using Cronbach's alpha, Lin's concordance, and Pearson or Spearman's correlation coefficients analyzing inner consistency, test-retest reliability, and association with other measures of HRQoL and health status. Categorical data were compared between groups using χ^2 test and continuous variables using t test or Kruskal-Wallis ANOVA. Discriminative power of the MARKO questionnaire was analyzed using receiver operator curve (ROC) analysis and presented with area under the curve (AUC) (with 95% confidence intervals [CI]) together with the associated criterion automatically calculated by statistical software, sensitivity, specificity, and positive (PPV) and negative predictive (NPV) values. For the main outcomes, logistic regression analysis with odds ratios (ORs) and 95% CIs for the 18-item MARKO questionnaire was calculated. $P<0.05$ was used as significant for all analyses with correction for multiple comparisons.

RESULTS

Pilot study

Results of the pilot study done in 138 COPD patients (52.1% women, 54.5 [10.7] years, 35.5 [25.5] pack-years) showed that all 18 items of the MARKO questionnaire had a comparable comprehension scores. The average score for each item was greater than 3.2 (out of maximum 4), meaning the items were easy to understand. The difference for comprehension between 18 items was not significant (Friedman ANOVA χ^2 [N = 134, df = 17] = 27.49, $P=0.051$).

Validation study

Men and women were of comparable age (52.0 vs 52.6 years, $P=0.537$) at the time of inclusion but men smoked significantly more (43.0 vs 32.2 pack-years, $P<0.001$) and were more likely to have quit ($P=0.012$), although most participants were current smokers (85.8%) (Table 1). More than half of the participants of both sex (men, 56.4% vs women, 56.1%, $P=0.840$) had chronic disorders other than respiratory and almost half of all participants were on some chronic disease treatment (42.7% vs 43.9%, $P=0.562$). Men had a significantly higher body mass index (27.5 vs 25.4 kg/m^2, $P<0.001$) with significantly higher systolic and diastolic blood pressure ($P=0.014$, $P=0.003$, respectively) and a comparable heart rate ($P=0.751$). Chronic or recurring respiratory symptoms were present in more than 60% of participants, with cough/sputum being present in approximately half of them and wheezing in more than 20%, with no significant difference between sexes ($P>0.300$ for all comparisons). No significant difference was found for FEV_1 ($P=0.620$) and FEV_1/FVC ratio ($P=0.066$) but men had significantly lower FVC ($P=0.001$) (Table 1).

Psychometric analyses indicated that the 18-item questionnaire had a very good internal consistency (Cronbach's alpha = 0.91) and test-retest reliability for a four week period (pc = 0.89, 95% CI 0.85-0.92, Lin's concordance; $r=0.89$, 95% CI 0.85-0.96, $P<0.001$, Pearson correlation).

The item-to-total correlations identified four questions whose coefficients were lower than 0.50, and the scores of this revised 14-item version of the questionnaire were also tested and compared with the scores of the 18-item questionnaire.

Internal consistency of the 14-item version was a bit better (Cronbach's alpha = 0.94), with a comparable test-retest re-

liability for a four week period (ρc = 0.88, 95% CI 0.84-0.91, Lin's concordance; r = 0.88, 95% CI 0.81-0.95, $P < 0.001$, Pearson correlation). The median (IQR) scores of the 18- and 14-item versions of the MARKO questionnaire, CAT scores, and SGRQ scores and subgroup comparisons are presented in Table 2. There were no significant differences in the scores of both versions for sex ($P > 0.200$ for both). The correlations of both scores with age were not significant ($r < 0.02$, $P > 0.800$ for both).

We found significant moderate positive correlations of MARKO scores with CAT scores of $r = 0.69$ (95% CI 0.59-0.79, $P < 0.001$) and $r = 0.63$ (95% CI 0.53-0.74, $P < 0.001$) for the 18- and 14-item versions, respectively. Comparable significant moderate positive correlations were found for the 18- and 14-item versions with the individual domains of SGRQ (Symptom score: $r = 0.69$, 95% CI 0.59-0.79, $P < 0.001$ and $r = 0.59$, 95% CI 0.48-0.71, $P < 0.001$, respectively; Activity score: $r = 0.67$, 95% CI 0.57-0.78, $P < 0.001$ and $r = 0.71$, 95% CI 0.61-0.81, $P < 0.001$, respectively; Impact score: $r = 0.68$, 95% CI 0.58-0.79, $P < 0.001$ and $r = 0.68$, 95% CI 0.57-0.78, $P < 0.001$, respectively). Strong positive correlations were found between MARKO scores and SGRQ total score ($r = 0.81$, 95% CI 0.73-0.89, $P < 0.001$ for the 18-item version; $r = 0.80$, 95% CI 0.72-0.88, $P < 0.001$ for the 14-item version).

Although analysis of variance was significant for between group comparisons ("healthy" smokers, symptomatic smokers, COPD GOLD 1, and COPD GOLD 2) for all HRQoL questionnaires (MARKO, CAT, and SGRQ, $P < 0.001$ for all), only the 18-item MARKO questionnaire showed a significantly lower median score in "healthy" smokers compared to other three groups (M = 7 vs 13 vs 10 vs 18, $P < 0.001$, $P = 0.045$ and $P < 0.001$, respectively; Table 2). Post-hoc analysis did not show a significant difference between other three groups ($P > 0.200$ for all comparisons for all HRQoL questionnaires). ROC curve analysis showed an AUC of 0.753 (95% CI 0.691 to 0.808, $P < 0.001$) with a sensitivity of 71.83% and specificity of 64.24%, PPV 48.57%, and NPV 82.91% for the MARKO score criterion of ≤10 for "healthy" smokers. Using "healthy" smokers as the reference group, 18-item MARKO questionnaire showed an OR of 1.14 (95% CI 1.08 to 1.20) for symptomatic smokers, OR of 1.10 (95% CI 1.03 to 1.18) for COPD GOLD 1, and OR of 1.17 (95% CI 1.09 to 1.26) for COPD GOLD 2 for each additional point on the scale ($P < 0.001$ for all).

TABLE 1. Characteristics of participants recruited in a validation study (N = 224)*†

Characteristics	Total (N = 224)	Men (n = 110)	Women (n = 114)	P
Age (years)	52.3 ± 6.7	52.0 ± 6.9	52.6 ± 6.4	0.537
Smoking history (pack-years)	37.5 ± 16.7	43.0 ± 17.9	32.2 ± 13.6	<0.001
Ex-smokers	32 (14.2)	22 (21.2)	10 (9.0)	0.012
Comorbidities	126 (56.3)	62 (56.4)	64 (56.1)	0.840
Chronic treatment	97 (43.3)	47 (42.7)	50 (43.9)	0.562
Body mass index (kg/m²)	26.4 ± 4.1	27.5 ± 3.9	25.4 ± 4.1	<0.001
Systolic blood pressure (mmHg)	126 ± 15	128 ± 14	123 ± 16	0.014
Diastolic blood pressure (mmHg)	80 ± 9	82 ± 9	78 ± 9	0.003
Heart rate (min⁻¹)	80 ± 12	79 ± 13	80 ± 11	0.751
Respiratory symptoms	138 (61.6)	66 (60.0)	72 (63.2)	0.627
wheezing	49 (21.9)	23 (20.9)	26 (22.8)	0.731
cough	114 (50.9)	54 (49.1)	60 (53.1)	0.550
sputum	107 (47.8)	53 (48.2)	54 (47.4)	0.903
night awakenings	16 (7.1)	10 (10.3)	6 (6.3)	0.307
chest pain	25 (11.2)	11 (10.2)	14 (12.6)	0.572
Respiratory sounds				
soft	73 (32.6)	43 (39.1)	30 (26.3)	0.027
prolonged expiration	25 (11.2)	13 (11.8)	12 (10.5)	0.949
rhonchi	30 (13.4)	15 (13.6)	15 (13.2)	0.908
Lung function (post bronchodilator)*				
FVC (% expected)	109.8 ± 16.9	106.0 ± 15.0	113.4 ± 17.9	0.001
FEV_1 (% expected)	99.7 ± 15.2	99.1 ± 14.8	100.2 ± 15.6	0.620
FEV_1/FVC (%)	76.0 ± 6.4	75.2 ± 6.7	76.8 ± 6.0	0.066

*FVC – forced vital capacity, FEV_1 – forced expiratory volume in 1 second.
†All data are presented as mean±SD or as number (%). Statistical significance for between sex comparisons was tested using *t* test or χ^2-test.

TABLE 2. Scores for the MARKO questionnaire, COPD Assessment Test (CAT) and St' George Respiratory Questionnaire (SGRQ) according to different subgroups*†

		MARKO questionnaire		CAT	SGRQ scores			
		18-item	14-item	score	symptom	activity	impact	total
All (N = 224)		11 (7-18.5)	8 (3.5-13.5)	8 (4-13)	14.5 (6.3-31.8)	18.3 (6-35.5)	3.9 (0-12.4)	12.5 (4.3-21.2)
Range		0-44	0-36	0-37	0-100	0-79.6	0-55.3	0-56.5
Sex	Men (n = 110)	10 (7-17)	7 (3-13)	8 (4-12)	16.0 (4.6-30.2)	23.2 (6-32.4)	4.2 (0-14.1)	12.9 (4.4-21.8)
	Women (n = 114)	13 (6-19)	9 (4-14)	9 (4-14)	13 (7.5-34.4)	17.4 (6-35.5)	3.7 (0-9.5)	11.3 (4.1-20)
	P	0.162	0.18	0.147	0.805	0.975	0.361	0.625
Subgroups after diagnostic workup	HS (n = 72)	7 (3-11)	5 (2-9)	5 (2-8)	6.3 (0-14.2)	11.2 (0-29.3)	0 (0-4)	5.2 (1.9-13.3)
	SS (n = 110)	13 (9-20)‖	9 (4-15)‖	10 (6-15)‖	19.6 (11-37.3)‖	23.4 (6-35.6)	6.7 (0-13.4)‖	14.7 (6.8-24.1)‖
	COPD GOLD 1 (n = 23)	10 (8-20)‡	8 (5-14)	9 (4-12)	11.4 (2.6-28)	20.4 (11.2-29.5)	5.1 (0-17.2)	12.3 (3.8-21.9)
	COPD GOLD 2 (n = 19)	18 (10-26)¶	13 (7-23)¶	11.5 (6.5-18)¶	29.2 (15.1-38.7)¶	23.3 (17.4-47.7)	11.4 (3-20.8)‡	18.3 (12.1-30.1)¶
	P	<0.001	<0.001	<0.001	0.039	<0.001	<0.001	<0.001
COPD	no (n = 182)	11 (6-16)	8 (3-12)	8 (4-13)	14.1 (6.3-31.7)	17.4 (6-35.4)	3.7 (0-10.6)	11.2 (4.1-19.9)
	yes (n = 42)	14 (9-24)	10 (5.5-18.5)	9 (5-15)	22.6 (5.4-34.4)	23.3 (12.4-35.8)	7.6 (0-18)	17.2 (7.5-27)
	P	0.008	0.015	0.133	0.264	0.226	0.098	0.090
Smoking	ex-smokers (n = 32)	10 (6.5-19)	8.5 (4-13.5)	7 (3-12)	6.3 (0-16.6)	29.5 (11.8-35.6)	4.2 (0-12.9)	15.5 (4.8-21.7)
	active (n = 192)	11 (7-19)	8 (3-14)	8 (4-14)	16.6 (8.8-34.4)	17.4 (6-32.5)	3.9 (0-12.4)	12.5 (4.4-21.3)
	P	0.657	0.697	0.324	0.002	0.054	0.637	0.533
Comorbidities	no (n = 98)	10 (6-16)	7.5 (3.5-12)	8 (4-13)	14.8 (6.3-34.4)	17.4 (6-29.5)	3.8 (0-11.4)	12.6 (4.3-17.4)
	yes (n = 126)	11 (7-19)	8 (4-15)	8 (4-13)	14.1 (5.1-27.9)	23.3 (6.2-35.5)	4.2 (0-13.3)	12.3 (4.4-22)
	P	0.238	0.113	0.943	0.414	0.132	0.527	0.440
Chronic treatment	no (n = 127)	10 (6-17)	7 (3-12)	8 (4-12)	14.1 (6.3-31.7)	17.1 (6-29.5)	2 (0-10.2)	11 (3.8-17.4)
	yes (n = 97)	11 (8-19)	8 (5-15)	9 (5-14)	14.9 (6.3-34.2)	23.5 (11.2-35.6)	6.1 (0-14.3)	14.7 (5.8-22.4)
	P	0.085	0.040	0.124	0.908	0.022	0.026	0.026
Respiratory symptoms	no (n = 86)	7.5 (3-14)	6 (2-10)	5 (2-9)	6.3 (0-14.2)	11.5 (0-29.5)	0 (0-4.2)	5.2 (1.9-14.4)
	yes (n = 138)	14 (9-21)	9 (5-15)	10 (6-15)	22.5 (11.1-38)	23.3 (11.2-35.5)	7.2 (0-14.9)	15 (7.9-24.1)
	P	<0.001	<0.001	<0.001	<0.001	0.013	<0.001	<0.001
Wheezing	no (n = 175)	10 (6-15)	7 (3-12)	7 (4-11)	11 (2.3-22.4)	17.1 (6-29.5)	2 (0-9.5)	9 (3.8-17)
	yes (n = 49)	19 (13-25)	12 (9-18)	14 (10-18)	34.6 (22.6-45.8)	29.5 (18.5-41.3)	10.3 (4.3-18.1)	21.4 (14.1-27.4)
	P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Chronic/ cough sputum	no (n = 110)	9 (4-14)	6 (3-10)	7 (3-11)	8.9 (0-18.3)	17.1 (6-29.5)	0 (0-7.9)	7.6 (2.5-16.3)
	yes (n = 114)	14 (10-22)	9 (5-16)	10 (6-16)	22.9 (11.1-40.5)	23.4 (11.2-35.6)	7.4 (0-15.9)	15.7 (6.9-25.4)
	P	<0.001	<0.001	<0.001	<0.001	0.017	<0.001	<0.001
Night awakenings	no (n = 208)	11 (6-18)	8 (4-12)	8 (4-12)	12.2 (6.3-30.2)	18.2 (6.2-35.4)	3.8 (0-10.4)	12.5 (4.4-19.4)
	yes (n = 16)	23 (12-34.5)	15 (8-28.5)	17 (13-25.5)	46.9 (22.9-64.7)	26.5 (12.2-53.6)	20.9 (12.5-36.6)	27 (21.9-45.7)
	P	<0.001	0.003	<0.001	<0.001	0.076	<0.001	<0.001
Chest pain	no (n = 199)	10 (6-15)	7 (3-12)	8 (4-12)	11.9 (4.4-28)	17.4 (6-29.5)	3.7 (0-10.3)	11.1 (4.1-18.2)
	yes (n = 25)	20 (15-27)	16 (11-20)	13 (9-18)	31.7 (15.3-49.4)	41.8 (23.7-53.6)	12.2 (3.6-25.7)	25.3 (15.7-36.5)
	P	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	<0.001
Fatigue	no (n = 160)	9 (4-13)	6 (3-9)	6.5 (3-10)	11.1 (2.6-27.3)	12.2 (0-23.5)	1.8 (0-7.4)	7.7 (3.6-14.7)
	yes (n = 64)	19 (14-25)	14.5 (11-20)	13 (8-18)	21.7 (9.6-40.5)	35.8 (23.7-48)	13.7 (7.1-22.9)	23.1 (17-30.1)
	P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Soft noise on auscultation	no (n = 151)	11 (6-16)	8 (3-12)	8 (4-13)	14.1 (6.6-27.3)	17.4 (6-29.5)	3.8 (0-11.4)	11.7 (4.5-18.6)
	yes (n = 73)	11 (6-19)	8 (4-13)	8 (4-13)	11.7 (2.3-34.4)	12.4 (0-35.8)	3.9 (0-15.4)	11.1 (3.6-26)
	P	0.786	0.881	0.744	0.745	0.824	0.750	0.916
Prolonged expiration	no (n = 199)	10 (6-16)	7 (3-12)	8 (4-13)	11.6 (4.5-28)	17.4 (6-35.4)	3.9 (0-11.4)	11.2 (4-19.6)
	yes (n = 25)	16 (9-25)	10 (6-21)	9.5 (5.5-14)	21.1 (10.7-34.4)	23.7 (12.4-35.3)	7.4 (0-18.7)	17.1 (5.2-27.4)
	P	0.007	0.008	0.194	0.076	0.226	0.122	0.080

TABLE 2. Continued. Scores for the MARKO questionnaire, COPD Assessment Test (CAT) and St' George Respiratory Questionnaire (SGRQ) according to different subgroups*†

		MARKO questionnaire		CAT	SGRQ scores			
		18-item	14-item	score	symptom	activity	impact	total
Rhonchi	no (n = 194)	10 (6-15.5)	7 (3-12)	8 (4-12)	11.6 (3.6-27.6)	17.4 (6-35.5)	3.8 (0-10.6)	11.3 (3.8-19.6)
	yes (n = 30)	18.5 (11-25)	12 (7-19)	12.5 (5.5-17)	23.8 (12.2-42.9)	18.5 (6-35.9)	10.3 (0-18.7)	13.7 (8-27.6)
	P	<0.001	0.004	0.021	0.001	0.581	0.025	0.031

*HS – "healthy" smokers/ex-smokers, SS – symptomatic smokers/ex-smokers, COPD GOLD 1 – participants diagnosed with chronic obstructive pulmonary disease (COPD) with Tiffeneau index <0.7 and forced expiratory volume in 1 second (FEV_1)>80% predicted, COPD GOLD 2 – participants diagnosed as COPD with Tiffeneau index <0.7 and FEV_1 <80% and ≥50% predicted.
†All data are presented as median and interquartile range (IQR) and as range for the overall scores. Statistical significance for subgroups comparisons was tested using Mann-Whitney U test for all independent variables except for 4 subgroups according to diagnosis after the diagnostic workup that was tested using Kruskal-Wallis ANOVA.
‡Significantly different from HS (post-hoc analysis): $P < 0.05$.
§Significantly different from HS (post-hoc analysis): $P < 0.01$.
‖Significantly different from HS (post-hoc analysis): $P < 0.001$.

The 14-item MARKO questionnaire, CAT, and SGRQ did not show significantly different scores between "healthy" smokers and COPD GOLD 1 subgroups ($P > 0.05$ for all comparisons; Table 2). Also the 18- and 14-item MARKO questionnaires were the only that significantly discriminated COPD from non-COPD participants (M = 14 vs 11, $P = 0.008$; 10 vs 8, $P = 0.015$; Table 2). ROC curve analysis for the 18-item MARKO questionnaire showed an AUC of 0.634 (95% CI 0.567 to 0.698, $P = 0.004$), with a sensitivity of 62.50% and specificity of 49.45%, PPV 21.37%, and NPV 85.71% for the score criterion of >10 for COPD. With each additional point on the scale of the 18-item MARKO questionnaire, the odds for COPD diagnosis significantly increased by 5% (OR 1.05, 95% CI 1.01 to 1.08, $P = 0.009$). AUC for the 14-item MARKO questionnaire was 0.623 (95% CI 0.555 to 0.687, $P = 0.010$). Although the scores for other questionnaires were lower in non-COPD participants, these differences were not significant ($P > 0.09$ for all).

Active smokers were significantly different from ex smokers only in the SGRQ symptoms domain (M = 16.6 vs 6.3, $P = 0.001$; Table 2). Having a comorbidity did not produce a significantly different score on any of the used questionnaires ($P > 0.110$), but using a chronic treatment for other than respiratory disorder produced a significantly different scores for the 14-item MARKO questionnaire (M = 8 vs 7, $P = 0.040$), SGRQ total score (M = 14.7 vs 11, $P = 0.026$), SGRQ activity domain (M = 23.5 vs 17.1, $P = 0.022$), and SGRQ impact domain (6.1 vs 2, $P = 0.026$; Table 2).

All four questionnaires significantly discriminated (Table 2) between the subgroups with or without chronic respiratory symptoms ($P < 0.001$ for all comparisons), with or without wheezing ($P < 0.001$ for all comparisons), with or without chronic cough and sputum ($P < 0.001$ for all comparisons), with or without night awakening ($P < 0.01$ for all comparisons), with or without chest pain ($P < 0.001$ for all comparisons), with or without fatigue ($P < 0.001$ for all comparisons), and with or without rhonchi during auscultation of lungs ($P < 0.05$ for all comparisons). ROC curve analysis for the 18-item MARKO questionnaire showed an AUC of 0.873 (95% CI 0.821 to 0.914, $P < 0.001$) with a sensitivity of 100% and specificity of 47.70%, PPV 44.14%, and NPV 100% for the score criterion of >8 for fatigue. None of the questionnaires (Table 2) significantly discriminated participants with a soft noise compared to normal noise during auscultation ($P > 0.740$), but the 18- and 14-item MARKO questionnaires showed a significantly different scores for the prolonged expiration (M = 16 vs 10, $P = 0.007$; 10 vs 7, $P = 0.008$; respectively). ROC curve analysis for the 18-item MARKO questionnaire showed an AUC of 0.667 (95% CI 0.596 to 0.731, $P = 0.004$), with a sensitivity of 56.00% and specificity of 70.62%, PPV 21.21%, and NPV 91.91% for the score criterion of >14 for prolonged expiration.

DISCUSSION

The main result of our initial validation study was that the MARKO questionnaire showed expected properties in a setup and population of the intended use (8). It was validated for comprehension and had a very good internal consistency and test-retest reliability, with high convergent validity correlation with the already validated COPD HRQoL questionnaires (SGRQ and CAT). A very important finding was that MARKO questionnaire better detected early symptoms in smokers than the other two questionnaires, significantly discriminating symptomatic smokers/ex-smokers and COPD patients from "healthy"

smokers/ex-smokers. Almost no differences were seen between the 14- and 18- item versions of the MARKO questionnaire, with a significantly better result for the 18-item version only regarding discriminating other subgroups from "healthy" smokers/ex-smokers. These results represent the first step and a prerequisite for further validation of the MARKO questionnaire regarding its predictive power as an early marker of future development of COPD (as a single marker or in combination) that can be used for screening in a primary care setting.

Population screening for COPD is not a recommended strategy but early diagnosis in a population at risk is highly recommended because of a high proportion of undiagnosed or late diagnosed COPD associated with high morbidity (1,2,9). Several approaches for use in primary care were tested but only to make an early diagnosis of the already present COPD (10). The MARKO questionnaire showed comparable results regarding the diagnostic potential for COPD in a primary care setting to the results of a meta-analysis of COPD Diagnostic Questionnaire (CDQ) by Haroon et al (10). However, rather than constructing a diagnostic questionnaire for COPD, our aim was to construct a questionnaire that could identify early changes in HRQoL in smokers leading to subsequent development of COPD. Having such an instrument could help in starting secondary prevention earlier or starting an early intervention. In regard to this aim, the MARKO questionnaire showed a higher sensitivity for early symptoms of future possible COPD than SGRQ or CAT, with high convergent validity correlation with these already validated COPD health status questionnaires. This high convergent validity correlation is also important because it shows specificity for respiratory disorders and could probably mean that it could be associated with already known features of CAT and SGRQ, showing association with many facets of COPD, like underlying inflammation, airway limitation, breathlessness, progression of disease, morbidity, and mortality (11-15). On the other hand, at least for the 18-item version, the results were not influenced by common comorbidities and concomitant treatment. In the systematic review by Haroon et al, the major risk for bias when evaluating the questionnaires and handheld flow meters for screening purposes was inadequate blinding between index tests and spirometry, which was not the case in our study (10).

Further validation is expected after a follow-up of the cohort of smokers recruited into the MARKO study, when the potential of this tool to predict future development of COPD in smokers/ex-smokers at risk for COPD will be evaluated (as a single tool or combined with other markers).

Based on basic psychometric analyses and high convergent validity correlation with already validated HRQoL questionnaires, the newly developed MARKO questionnaire was shown to be a reliable self-administered short health status assessment tool. It had a better discriminating power for early changes associated with smoking susceptibility than other two questionnaires (CAT and SGRQ), thus being in accordance with the newest recommendations as a first step in making an early diagnosis. These properties will be tested prospectively in an ongoing cohort study to evaluate the predictive power of the MARKO questionnaire to identify individuals who will develop COPD among individuals at risk.

Acknowledgments MARKO group: ***Principal Investigators:*** Assoc.Prof. Davor Plavec, MD, MSc, PhD, Children's Hospital Srebrnjak, Zagreb and Faculty of Medicine JJ Strossmayer University, Osijek, Croatia; Žarko Vrbica, MD, MSc, General Hospital Dubrovnik and University of Dubrovnik, Dubrovnik, Croatia. ***Consultant:*** Prof. Peter MA Calverley, University of Liverpool, Liverpool, UK; ***Psychologists:*** Assist.Prof. Adrijana Košičec Đuknić, PhD, University of Zagreb Centre for Croatian Studies, Zagreb, Croatia; Assist.Prof. Biserka Radoševič-Vidaček, BA, MA, PhD, Institute for Medical Research and Occupational Health, Zagreb, Croatia. ***Pulmonologists:*** Elvo Ljubičić, MD, University Hospital Dubrava, Zagreb, Croatia; Iva Jurić, MD, University Hospital Center Osijek, Osijek, Croatia; Assist.Prof. Ivan Gudelj, MD, PhD, University Hospital Center Split, Split, Croatia; Assist.Prof. Ljiljana Bulat Kardum, MD, PhD, University Hospital Center Rijeka, Rijeka, Croatia; Marina Labor, MD, University Hospital Center Osijek, Osijek, Croatia; Assoc.Prof. Neven Tudorić, University Hospital Dubrava, Zagreb, Croatia; Assoc.Prof. Sanja Popović Grle, MD, PhD, University Hospital Center Zagreb, Zagreb, Croatia; Slavica Labor, MD, PhD, University Hospital Center Osijek, Osijek, Croatia; Tajana Jalušić Glunčić, MD, University Hospital Center Zagreb, Zagreb, Croatia; Tina Lukić, MD, Special Hospital for Lung Diseases, Zagreb, Croatia. ***General practice:*** Albina Dumić, MD, PhD, Family Physician's Office, Osijek, Croatia; Alen Stojanović, MD, Family Physician's Office, Osijek, Croatia; Bojana Vakanjac, MD, General Practitioner's Office, Osijek, Croatia; Darja Nelken-Bestvina, MD, General Practitioner's Office, Osijek, Croatia; Davorka Martinković, MD, Family Physician's Office, Zagreb, Croatia; Dubravka Margaretić, MD, General Practitioner's Office, Osijek, Croatia; Ines Balint, MD, General Practitioner's Office, Strmec Samoborski, Croatia; Assoc.Prof. Ines Diminić Lisica, MD, PhD, Family Physician's Office, Rijeka, Croatia; Ivana Boban, MD, General Practitioner's Office, Split, Croatia; Jadranka Radman, MD, General Practitioner's Office, Split, Croatia; Jasna Nagyszombaty-Šarić, MD, General Practitioner's Office, Osijek, Croatia; Karla Tudja, Family Physician's Office, Donji Muć, Croatia; Ljiljana Ismić, MD, Family Physician's Office, Osijek, Croatia; Ljiljana Lulić-Karapetrić, MD, Family Physician's Office, Zagreb, Croatia; Merim Bezdrov, MD, General Practitioner's Office, Split, Croatia; Mirjana Bezdrov, MD, General Practitioner's Office, Split, Croatia; Monika Jedud, MD, Family Physician's Office, Osijek, Croatia; Nataša Mrduljaš-Đujić, MD, Family Physician's Office, Postira, Croatia; Rosanda Rosandić Plasevoli, MD, General Practitioner's Office, Split, Croatia; Sanda Pribić, MD, Family Physician's Office, Osijek, Croatia; Renata Grgurić, MD, General Practitioner's Office, Osijek, Croatia; Suzana Maltar-Delija, MD, General Practitioner's Office, Zagreb, Croatia; Vanja Vlaić, MD, Family Physician's Office, Split, Croatia; Vjekoslava Amerl-Šakić, MD, Family Physician's Office, Zagreb, Croatia; Zdenka Meštrović, MD, General Practitioner's Office, Zagreb, Croatia.

Funding The study was supported by the unrestricted grant from GlaxoSmithKline (GSK eTrack number: CRT114338).

Ethical approval received from the Children's Hospital Srebrnjak Ethics Committee.

Declaration of authorship ZV participated in conceiving the study, its design and coordination, and drafting and revising of the manuscript for important intellectual content. DP participated in conceiving the study, its design and coordination, data acquisition, statistical analysis, and drafting and revising the manuscript for important intellectual content. BRV participated in conceiving the study, its design and coordination, and drafting and revising the manuscript for important intellectual content. AKD participated in conceiving the study, its design and coordination, and drafting and revising the manuscript for important intellectual content. ML participated in study coordination, data acquisition, and drafting and revising the manuscript for important intellectual content. IG participated in study coordination, data acquisition, and drafting and revising the manuscript for important intellectual content. SL participated in study coordination, data acquisition, and drafting and revising the manuscript for important intellectual content. IJ participated in study coordination, data acquisition, and drafting and revising the manuscript for important intellectual content. PMAC participated in conceiving the study, its design and coordination, and drafting and revising the manuscript for important intellectual content. All authors contributed to the original ideas and gave the final approval for the final version of the manuscript.

Competing interests All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: no support from any organization for the submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years; no other relationships or activities that could appear to have influenced the submitted work.

References

1 Global Strategy for the Diagnosis, Management and Prevention of COPD, Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2016. Available from: http://www.goldcopd.org/. Accessed: October 26, 2016.

2 Nardini S, Annesi-Maesano I, Del Donno M, Delucchi M, Bettoncelli G, Lamberti V, et al. The AIMAR recommendations for early diagnosis of chronic obstructive respiratory disease based on the WHO/GARD model*. Multidiscip Respir Med. 2014;9:46. Medline:25473523 doi:10.1186/2049-6958-9-46

3 Quanjer PH, Stanojevic S, Cole TJ, Baur X, Hall GL, Culver BH, et al; ERS Global Lung Function Initiative. Multi-ethnic reference values for spirometry for the 3-95 years age range: the Global Lung Function 2012 equations. Eur Respir J. 2012;40:1324-43. Medline:22743675 doi:10.1183/09031936.00080312

4 Wain LV, Shrine N, Miller S, Jackson VE, Ntalla I, Artigas MS, et al. Novel insights into the genetics of smoking behaviour, lung function, and chronic obstructive pulmonary disease (UK BiLEVE): a genetic association study in UK Biobank. Lancet Respir Med. 2015;3:769-81. Medline:26423011 doi:10.1016/S2213-2600(15)00283-0

5 Jones PW, Quirk FH, Baveystock CM, Littlejohns P. A self-complete measure for chronic airflow limitation - the St George's Respiratory Questionnaire. Am Rev Respir Dis. 1992;145:1321-7. Medline:1595997 doi:10.1164/ajrccm/145.6.1321

6 Jones PW, Harding G, Berry P, Wiklund I, Chen WH, Kline Leidy N. Development and first validation of the COPD Assessment Test. Eur Respir J. 2009;34:648-54. Medline:19720809 doi:10.1183/09031936.00102509

7 Beaton DE, Bombardier C, Guillemin F, Ferraz MB. Guidelines for the process of cross-cultural adaptation of self-report measures. Spine. 2000;25:3186-91. Medline:11124735 doi:10.1097/00007632-200012150-00014

8 Ställberg B, Nokela M, Ehrs PO, Hjemdal P, Jonsson EW. Validation of the clinical COPD Questionnaire (CCQ) in primary care. Health Qual Life Outcomes. 2009;7:26. Medline:19320988 doi:10.1186/1477-7525-7-26

9 Celli BR. Recommendations for the early diagnosis of COPD: the AIMAR view. Multidiscip Respir Med. 2015;10:6. Medline:25729573 doi:10.1186/s40248-015-0005-4

10 Haroon S, Jordan R, Takwoingi Y, Adab P. Diagnostic accuracy of screening tests for COPD: a systematic review and meta-analysis. BMJ Open. 2015;5:e008133. Medline:26450427 doi:10.1136/bmjopen-2015-008133

11 Sarioglu N, Hismiogullari AA, Bilen C, Erel F. Is the COPD assessment test (CAT) effective in demonstrating the systemic inflammation and other components in COPD? Rev Port Pneumol. 2016;22:11-7. Medline:26534740

12 Wilcox TK, Chen WH, Howard KA, Wiklund I, Brooks J, Watkins ML, et al. Item selection, reliability and validity of the Shortness of Breath with Daily Activities (SOBDA) questionnaire: a new outcome measure for evaluating dyspnea in chronic obstructive pulmonary disease. Health Qual Life Outcomes. 2013;11:196. Medline:24229361 doi:10.1186/1477-7525-11-196

13 Miravitlles M, García-Sidro P, Fernández-Nistal A, Buendía MJ, Espinosa de los Monteros MJ, Molina J. Course of COPD assessment test (CAT) and clinical COPD questionnaire (CCQ) scores during recovery from exacerbations of chronic obstructive pulmonary disease. Health Qual Life Outcomes. 2013;11:147. Medline:23987232 doi:10.1186/1477-7525-11-147

14 Miravitlles M, García-Sidro P, Fernández-Nistal A, Buendía MJ, Espinosa de Los Monteros MJ, Esquinas C, et al. The chronic obstructive pulmonary disease assessment test improves the predictive value of previous exacerbations for poor outcomes in COPD. Int J Chron Obstruct Pulmon Dis. 2015;10:2571-9. Medline:26664105 doi:10.2147/COPD.S91163

15 Sundh J, Janson C, Lisspers K, Montgomery S, Ställberg B. Clinical COPD Questionnaire score (CCQ) and mortality. Int J Chron Obstruct Pulmon Dis. 2012;7:833-42. Medline:23277739 doi:10.2147/COPD.S38119

3.3. Diagnostic accuracy of a pocket screening spirometer in diagnosing chronic obstructive pulmonary disease in general practice: a cross sectional validation study using tertiary care as a reference.

Labor et al. BMC Family Practice (2016) 17:112
DOI 10.1186/s12875-016-0518-8

BMC Family Practice

RESEARCH ARTICLE Open Access

Diagnostic accuracy of a pocket screening spirometer in diagnosing chronic obstructive pulmonary disease in general practice: a cross sectional validation study using tertiary care as a reference

Marina Labor[1,2†], Žarko Vrbica[3,4†], Ivan Gudelj[5], Slavica Labor[1,2] and Davor Plavec[2,6*]

Abstract

Background: COPD-6™ is a lung function testing device for a rapid pre-spirometry testing to screen-out at-risk individuals not having COPD and indicating those at risk. The aim of this study was to validate COPD-6™ lung function testing (index test) in general practice in discriminating patients with COPD out of the population at risk - smokers/ex-smokers with no previous diagnosis of COPD, using measurements at tertiary care as reference standard.

Methods: Consecutive 227 subjects (115 women, 185 smokers/42 ex-smokers, ≥20 pack-years) with no previous diagnosis of COPD, aged 52.5 (SD 6.8) years from 26 general practitioners (GPs) were recruited, lung function tested with COPD-6™, referred to the tertiary institution for repeated COPD-6™ testing followed by spirometry with a bronchodilator (salbutamol), examination, and pulmonologist consultation for the diagnosis and severity of COPD.

Results: COPD was diagnosed in 43 subjects (18.9 %), with an AUC of 0.827 (95 % CI 0.769-0.875, $P < 0.001$) for the diagnosis of COPD when lung function was measured using COPD-6™ in GP's office with a specificity of 100 % (95 % CI, 97.95–100 %) but a very low sensitivity of 32.56 % (95 % CI, 20.49–47.48 %). Significant agreement for forced expiratory volume in 1 s measured at GP's office and at lung function lab was found (mean difference 0.01 L, $p = 0.667$) but not for other measured parameters ($p < 0.001$ for all).

Conclusions: Our study results point out that active case finding in a population at risk for COPD should be instituted (almost 20 % of undiagnosed COPD). Based on our results lung function testing with COPD-6™ can substitute spirometry testing in cases where it is not readily available to the patient/physician taken into account that the traditional FEV_1/FEV_6 cutoff value of <0.7 is not the only criterion for diagnosis and/or further referral.

Trial registration: ClinicalTrials.gov Identifier NCT01550679 Registered 28 September 2014, retrospectively registered

Keywords: COPD, Diagnosis, General practice, Screening, Sensitivity and specificity

Abbreviations: ANOVA, Analysis of variance; ATS, American Thoracic Society; AUC, Area under the curve; COPD, Chronic obstructive pulmonary disease; ERS, European Respiratory Society; FEV_1, Forced expiratory volume in 1. second;

(Continued on next page)

* Correspondence: plavec@bolnica-srebrnjak.hr
†Equal contributors
[2]Faculty of Medicine, J.J. Strossmayer University of Osijek, Ulica cara Hadrijana 10E, Osijek, Croatia
[6]Research Department, Children's Hospital Srebrnjak, Srebrnjak 100, Zagreb, Croatia
Full list of author information is available at the end of the article

(Continued from previous page)
FEV_6, Forced expiratory volume in 6 s; FVC, Forced vital capacity; GOLD, Global initiative for chronic obstructive lung disease; GP, General practitioner; LLN, Lower limit of normal; NPV, Negative predictive value; PPV, Positive predictive value; QoL, Quality of life; ROC, Receiver operator curve; SD, Standard deviation

Background

COPD is one of the leading causes of morbidity and mortality worldwide [1]. Existing prevalence data show variations due to survey methods reflecting also a widespread under-diagnosis of COPD, even in patients with developed respiratory symptoms [2–5]. On the other hand patients with a mild COPD already have substantial reduction in all parameters of health related QoL (HRQoL) [6]. Modern strategies for COPD management are stressing the importance of primary care physician's office-based assessments of patients at risk, thus significantly increasing the number of timely diagnosed COPD patients [7].

Spirometry with the documented post-bronchodilator FEV_1/FVC <0.70 is required to make a diagnosis of COPD in a clinical context of the disease [1]. Spirometry in general practitioners' (GPs) office would be adequate to make a diagnosis, but there are many obstacles to that strategy; the price of equipment, reimbursement strategies, quality of spirometry in such an environment, insufficient training, and experience in testing [8]. Unavailability of a spirometry often leads to overdiagnosis of COPD, made only based on symptoms and exposure data without confirmatory lung function testing, leading to overtreatment and negative impact on morbidity and mortality [9–11].

COPD-6™ (4000 COPD-6™ Respiratory Monitor, Vitalograph Ltd., Buckingham, UK) is a cheap, simple lung function testing device approved as a rapid pre-spirometry testing tool to screen-out the at-risk individuals who do not have COPD and indicate those that may be at risk. It is a simple device, easy to learn how to operate, having the readout without the risk of false COPD negatives, thus focusing spirometry resources on a smaller population with most of the risk. Four major problems could arise from using COPD-6™ as a screening device instead of spirometry: (1) using FEV_6 instead of FVC could underestimate the later; (2) no post-bronchodilator testing; (3) no flow-volume curve presentation; (4) a single criterion for fixed airflow limitation as defined in GOLD initiative ($FEV_1/FEV_6 < 0.70$) thus possibly producing a significant over-diagnosis in elderly (>70 years of age) according to The Global Lung Function Initiative data [12].

Having all that in mind, we wanted to explore the diagnostic accuracy of COPD-6™ in a population of smokers/ex-smokers with a significant exposure to cigarette smoke, with no previous diagnosis of COPD, in a general practice setting comparing it to the 'gold standard', spirometry conducted in a lung function laboratory at the tertiary care level (university and teaching hospitals) by experienced staff with special training. This is a population with expected 25 % of undiagnosed COPD cases in which such case identification is recommended [1]. So, the aim of this study was to validate COPD-6™ lung function testing (index test) in general practice in discriminating patients with COPD out of the population at risk - smokers/ex-smokers with no previous diagnosis of COPD, using measurements at tertiary care as reference standard. The secondary goal was to assess the agreement between lung function measurements between methods (COPD-6™ vs. spirometry) and between health care settings (primary vs. tertiary care).

Methods

Study framework

This prospective cohort study was a part of broader research project (Early detection of COPD patients in GOLD 0 (smokers) population – MARKO project). The whole protocol of the MARKO project can be found at https://clinicaltrials.gov/ct2/show/NCT01550679. The study was approved by the local ethics committee and conducted according to the Declaration of Helsinki and other relevant international and national laws. The patients were approached by their GPs during any (unrelated to respiratory problems) visit to their office if they were smokers or ex-smokers of the predefined age group for the study together with the prescreening for inclusion/exclusion criteria using a structured interview. Eligible patients were given the Informed consent document with enough time to read it and to discuss any relevant issues regarding the study before they signed the written consent. They were informed about the prospective nature of the study and their right to withdraw their consent and claim the withdrawal of all gathered data and destroying all biological samples at any time without any explanation, obligation or consequence from their side. All participants signed the written consent before starting any procedure for the study.

Subjects

The consecutive patients from 26 GPs (representing the same number of GP offices) were recruited based on inclusion/exclusion criteria. We decided on consecutive patients based on the limited number of insured persons under the care by each GP (approx. 1700), and relatively

low response rate for a public health campaigns in our country. Inclusion criteria were: written consent; smokers/ex-smokers of either sex, aged 40–65 years with a smoking history of at least 20 pack-years (calculated as number of cigarettes smoked per day multiplied by the number of years of smoking divided by 20); with no previous diagnosis of COPD. Exclusion criteria were: any clinically relevant chronic disease significantly affecting QoL at the time of the first visit (cardiovascular, cerebrovascular, diabetes, hepatitis, nephropathy, chronic dialysis, systemic disorder, cancer); immunosuppressive therapy; preceding acute respiratory disease 4 weeks before the visit; hospitalization for any reason during past 3 months; myocardial infarction, cerebrovascular infarction or transient ischemic attack during past 6 months; diagnosis of asthma; and an inability to perform the diagnostic protocol. Exclusion criteria were introduced not to exclude patients having comorbidities common in COPD, but to exclude the ones that represent acute/subacute clinical states/disorders or states of recovery from major clinical disorders representing an absolute or relative contraindication for spirometry or significantly influencing the diagnostic process or an already present diagnosis of respiratory disorder (e.g., asthma).

Study workup

All GPs went through short small groups training and were provided with the COPD-6™ devices. GPs were not extensively trained in spirometry or assessed for their skill level because we wanted that the measurements would be performed as close as possible to the regular real-life clinical situation where GPs scarcely use these measurements. After the examination at the GP's office and lung function testing with COPD-6™ (index test), patients were referred after 2–4 weeks to one of the tertiary institutions to a designated team consisting of a pulmonologist, research nurse and lung function laboratory technician. Standard diagnostic workup consisted of repeated COPD-6™ lung function testing followed by spirometry with a bronchodilator (salbutamol), history, physical examination and specialist consultation. Patients with no previous diagnosis of COPD were chosen to avoid bias coming from a previous knowledge of a diagnosis but allowing a significant subsample of subject with COPD (according to previous studies up to 25 % of subjects in this population has an undiagnosed COPD) [1]. To avoid the second possible bias, the team at the tertiary care institution was blinded for the results of the COPD-6™ measurements performed at GP's office. After the workup conducted at the tertiary care institution the pulmonologist made the diagnosis and severity assessment of COPD according to GOLD: relevant exposure, respiratory symptoms characteristic for COPD and fixed airflow limitation (post bronchodilator FEV_1/FVC <0.70) [1]. This was used as a reference standard for this study.

Lung function measurements

Lung function measurements using COPD-6™ were performed according to the manufacturer's recommendations and ATS/ERS guidelines [11]. Measurements were repeated until 3 technically satisfactory efforts were performed. COPD-6™ has a quality assessment built in the device and marks the technically inadequate measurement with the exclamation mark. Exclamation mark appears when the time of expiration is too short or coughing during expiration was present. After 3 technically satisfactory efforts the device automatically choses the best one and these results were recorded as absolute values for forced expiratory volume in 1 s (FEV_1 in L), forced expiratory volume in 6 s (FEV_6 in L), FEV_1/FEV_6 ratio (%), and lung age (years) and as % of predicted values for FEV_1, FEV_6 and FEV_1/FEV_6 according to prediction equations already in the device calculated according to sex, age and height. The device uses the prespecified cut-off levels for visually suggesting the preliminary diagnosis of COPD (FEV_1/FEV_6 ratio of <0.7) and assesses the severity according to GOLD initiative [1], so we used this criterion as a positive index test for further comparisons. The same procedure was followed at both sites (GP's offices and lung function labs in a tertiary care hospitals).

Spirometry was performed using computerized pneumotachographs (Jaeger®, CareFusion, CA, USA) using the same procedure at all clinical sites (lung function labs at tertiary hospitals) according to ATS/ERS guidelines [13]. The best of three technically satisfactory efforts was recorded. Bronchodilator test was performed by repeated spirometry 20 min after the inhalation of 400 mcg of salbutamol using the inhalation chamber. Absolute values of FEV_1, forced expiratory capacity (FVC), FEV_1/FVC ratio were together with sex, age and height entered into the Excel sheet (Microsoft® Excel® 2013, Microsoft Corporation, USA) for all subjects and using predicted values equation from Quanjer, % of predicted was calculated in a single act using Excel [14]. Tertiary care postbronchodilator spirometry measurements at lung function laboratory were used as reference standard for this study.

Data analyses

Data analysis was performed using STATISTICA version 12 (StatSoft, Inc., OK, USA) and MedCalc Statistical Software version 15.8 (MedCalc Software bvba, Ostend, Belgium; https://www.medcalc.org; 2015). Minimal sample size of 70 subjects (14 positive and 56 negative) was calculated for the expected area under the curve (AUC) of 0.8 with a statistical power

of 95 % (beta 0.05) and alpha of 0.05. Categorical data was presented as absolute and relative (%) numbers. Continuous variables were presented as mean and standard deviations (SD). Categorical data was compared between subgroups using chi-square ($\chi 2$) test and continuous variables using Student's *t*-test or Mann-Whitney *U* test and analysis of variance (ANOVA). The criteria to use Student's *t*-test and ANOVA were checked and fulfilled before the tests were performed. Agreement between lung function measurement methods was conducted using Bland-Altman statistics and plots. Utility of FEV_1/FEV_6 measured using COPD-6™ at GP's office (index test) for diagnosing COPD was analyzed comparing it to the reference standard using receiver operator curve (ROC) analysis and data was presented as AUC together with sensitivity, specificity and positive and negative predictive values together with 95 % confidence intervals (CIs). $P < 0.05$ was used as statistically significant for all analyses.

Results

Out of 326 consecutive prescreened smokers of eligible age and smoking history, 227 (69.6 %) subjects (115 women) at risk for COPD (185 smokers and 42 ex-smokers) aged 52.5 (SD 6.8) years were included in this study (78 refused to participate and 21 were excluded based on exclusion criteria, Fig. 1). The basic demographic data for included subjects are displayed in Table 1. The diagnosis of COPD (reference diagnosis) was made in 43 (18.9 %) subjects with no significant difference between men and women ($\chi 2 = 2.711$, $P = 0.100$) or between smokers and ex-smokers ($\chi 2 = 1.763$, $P = 0.185$). Cross-tabulation of an index test positivity against the reference standard is presented in Table 2. No significant difference was found between subjects with COPD and no-COPD for age ($t = 1.139$, $P = 0.256$), presence of comorbid disorders (55.5 %) and chronic treatment ($\chi 2 = 0.049$, $P = 0.825$; $\chi 2 = 0.125$, $P = 0.724$; respectively), BMI ($t = 0.100$, $P = 0.921$) or smoking habit ($p > 0.100$ for all parameters of smoking habit). Also no clustering

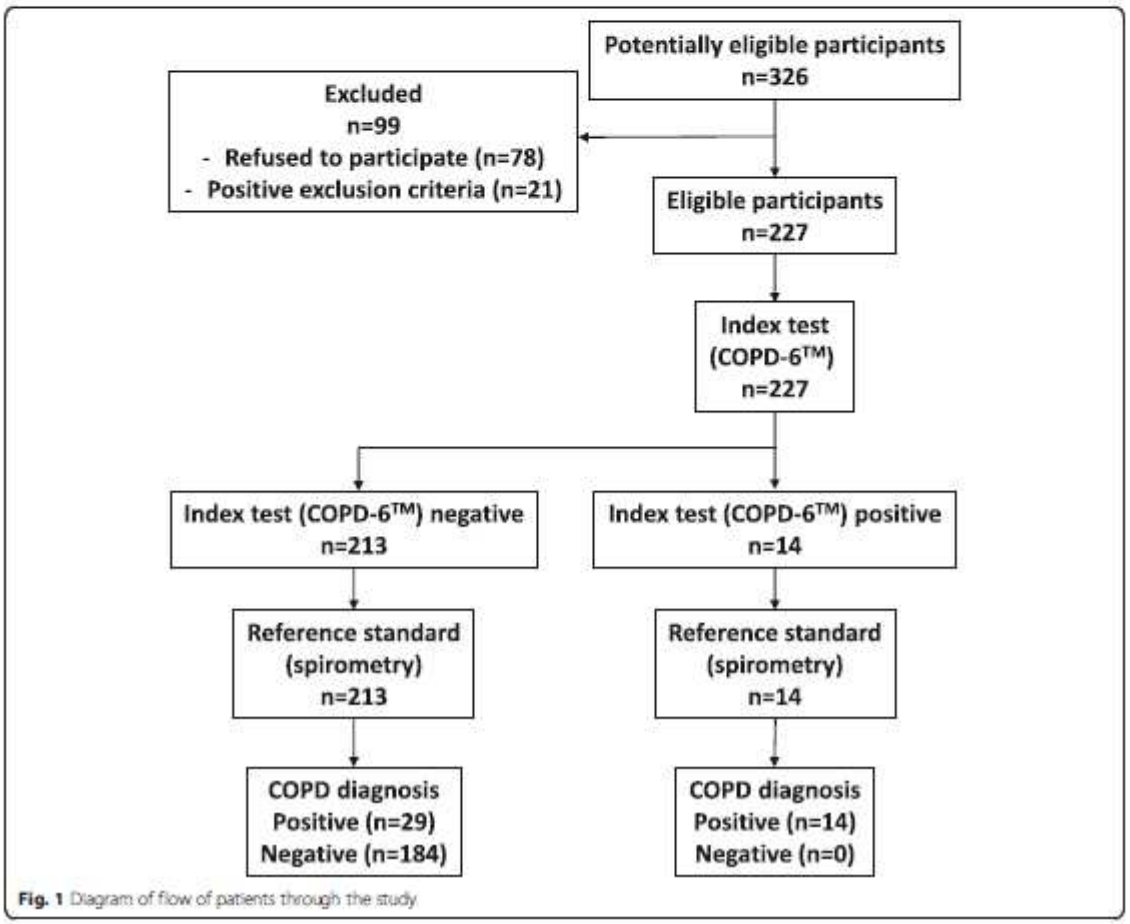

Fig. 1 Diagram of flow of patients through the study

Table 1 Demographics, smoking habit, presence of comorbid disorders and chronic treatment other than that for COPD according to final COPD diagnosis ($N = 227$)

Variables	All ($N = 227$)	COPD ($n = 43$)	Non-COPD ($n = 184$)	Statistics
Women (%)	115 (50.7)	17 (39.5)	99 (53.2)	$\chi2 = 2.711$, $P = 0.100$
Age (years), mean ± SD	52.5 ± 6.8	53.6 ± 7.0	52.3 ± 6.7	$t = 1.139$, $P = 0.256$
BMI (kgm^{-2}), mean ± SD	26.5 ± 4.2	26.5 ± 5.2	26.5 ± 3.9	$t = 0.100$, $P = 0.921$
Active smokers (%)	185 (84.9)	32 (84.2)	153 (85.0)	$\chi2 = 0.015$, $P = 0.902$
Years of smoking, mean ± SD	30.6 ± 6.9	32.0 ± 6.4	30.3 ± 6.9	$z = 1.641$, $P = 0.101$
Cigarettes/day, mean ± SD	24.6 ± 9.1	24.4 ± 8.0	24.6 ± 9.2	$z = 0.241$, $p = 0.809$
Pack-years, mean ± SD	37.9 ± 17.4	39.1 ± 14.3	37.5 ± 17.5	$z = 1.310$, $p = 0.190$
Presence of comorbid disorders (%)	126 (55.5)	22 (51.2)	104 (56.5)	$\chi2 = 0.049$, $P = 0.825$
Chronic treatment (%)	99 (43.6)	16 (37.2)	83 (45.1)	$\chi2 = 0.125$, $P = 0.724$

χ2 chi-square test results, *t* result of Student's *t*-test, *z* result of Mann-Whitney *U* test, *SD* standard deviation, *BMI* body mass index calculated as the ratio of body weight in kg and squared body height in meters

of COPD diagnosis, demographics or smoking habit data was evident for different GPs ($p > 0.100$ for all comparisons). In Table 3 lung function data is presented according to the existence and severity of COPD (24, 10.4 % GOLD stage 1 and 19, 8.2 % GOLD stage 2).

ROC curve analyses of FEV_1/FEV_6 measurements for the diagnosis of COPD using COPD-6™ at the GPs office and at the lung function lab at the tertiary care hospital gave an AUC of 0.827 (95 % CI 0.769–0.875, $P < 0.001$; Fig. 2) and 0.849 (95 % CI 0.788–0.898, $P < 0.001$) being significantly different to spirometry (AUC 0.961, 95 % CI 0.920–0.984, z statistic = 2.501, $P = 0.012$; z statistic = 4.058, $P < 0.001$; respectively). Using the usual (pre-specified) threshold of <0.7 for FEV_1/FEV_6 for the diagnosis of COPD for the lung function (COPD-6™) values measured at GP's offices gave the highest specificity of 100 % (95 % CI, 97.95–100 %) but a very low sensitivity of 32.56 % (95 % CI, 20.49–47.48 %) with a PPV of 100 % (95 % CI, 78.47–100 %) and NPV of 86.38 % (95 % CI, 81.13–90.35 %).

Exploratory analyses using the change in threshold gave somewhat better overall results with a change of threshold to ≤0.78 for FEV_1/FEV_6 that gave a specificity of 88.95 % (95 % CI, 83.83–92.60 %) with a sensitivity of 70.97 % (95 % CI, 54.72–85.03 %), a PPV of 52.38 % (95 % CI, 37.72–66.64 %), NPV of 94.71 % (95 % CI, 89.90–97.76 %). The highest NPV (95.74 %, 95 % CI 90.93–98.79 %) was achieved with a cut-off value of 0.85 with the negative likelihood value of 0.26 (95 % CI, 0.20–0.33).

Table 2 Cross-tabulation of the results of index test (COPD-6™) against the reference standard (spirometry) ($N = 227$)

Index test (COPD-6™)	Reference standard (spirometry)		
	Positive	Negative	Total
Positive	14	0	14
Negative	29	184	213
Total	43	184	227

Methods comparison between lung function values measured at GP's offices and values measured at lung function labs at tertiary care hospitals are shown in Table 4 and Fig. 3. FEV_1 values measured using COPD-6™ at GP's offices showed small differences with the same measurement at tertiary care and no clinically relevant differences when compared with spirometric measurement and postbronchodilator one (mean difference, −0.12 L and −0.16 L, $P < 0.001$ for both, Bland-Altman statistics; Table 4 and Fig. 3). Point of care comparison for FEV_6 (primary vs. tertiary care) showed clinically non-relevant difference (Table 4 and Fig. 3) but method comparison with spirometric and postbronchodilator measurements of FVC showed significant and clinically relevant differences (mean difference, −0.66 L and −0.60 L, $P < 0.001$ for both, Bland-Altman statistics) when compared to reference measures with a trend of increasing the difference with larger values (Table 4 and Fig. 3). Comparison of FEV_1/FEV_6 values measured using COPD-6™ showed significant and clinically relevant differences (mean difference, 4.2 %, 10.2 % and 8.6 %, $P < 0.001$ for all, Bland-Altman statistics) when compared to reference measures (FEV_1/FEV_6, FEV_1/FVC and postbronhodilator FEV_1/FVC) at tertiary care hospitals with a trend of increasing the difference with lower values (Table 4 and Fig. 3) thus showing a systematic bias.

Discussion

This study has three main findings: (1) COPD-6™ showed moderate accuracy with high specificity but low sensitivity for COPD; (2) COPD-6™ could be with certain restrictions reliably used in GP's offices; (3) in the population at risk for COPD, there was a substantial number (18.9 %) of undiagnosed patients. Our data comparing COPD-6™ measurements with a reference standard (tertiary care COPD diagnosis) shows that COPD-6™ can be

Table 3 Lung function (COPD-6™, spirometry) according to the presence and severity of COPD according to GOLD stages

	Lung function	All	Non-COPD	COPD GOLD 1	COPD GOLD 2	Statistics
		(*N* = 227)	(*n* = 184)	(*n* = 24)	(*n* = 19)	
COPD-6™	FEV_1 (% predicted)	94.3 ± 15.6	97.6 ± 13.3	90.9 ± 13.0	67.5 ± 12.2	*F* = 46.27 *P* < 0.001
	FEV_6 (% predicted)	93.9 ± 16.2	96.0 ± 15.3	94.7 ± 14.9	74.5 ± 13.7	*F* = 17.27 *P* < 0.001
	FEV_1/FEV_6 (%)	0.845 ± 0.085	0.864 ± 0.071	0.781 ± 0.083	0.757 ± 0.117	*F* = 25.84 *P* < 0.001
	Lung age (yrs)	60.7 ± 13.9	57.7 ± 11.1	64.0 ± 11.1	84.6 ± 15.7	*F* = 48.07 *P* < 0.001
Spirometry	FEV_1 (% predicted)	97.9 ± 15.3	101.5 ± 12.9	92.9 ± 10.2	71.1 ± 12.7	*F* = 51.50 *P* < 0.001
	FVC (% predicted)	109.3 ± 17.0	110.8 ± 16.7	112.7 ± 11.8	91.1 ± 16.2	*F* = 13.29 *P* < 0.001
	FEV_1/FVC (%)	0.742 ± 0.073	0.761 ± 0.060	0.665 ± 0.055	0.650 ± 0.073	*F* = 49.14 *P* < 0.001
	ΔFEV_1 (%)	1.39 ± 4.00	1.40 ± 3.79	2.92 ± 4.25	−0.93 ± 7.68	*F* = 3.011 *P* = 0.051

Data for all variables is presented as mean ± standard deviation; *FEV_1* forced expiratory volume in 1 s, *FEV_6* forced expiratory volume in 6 s, *FVC* forced expiratory volume, *ΔFEV_1* post-bronchodilator change in FEV_1 (measured 20 min after inhalation of 400 μg of salbutamol), *F* – result of ANOVA for between group comparisons

used with enough accuracy in screening for COPD on a primary care level. Exploratory analyses with the change in threshold showed the possible improvement in the accuracy, but this data needs additional confirmatory studies to be conducted using these values as a pre-specified ones. Although it is our opinion that based on substantial number of undiagnosed patients screening for COPD in a population at risk is valuable, this opinion needs further corroboration based on the studies arguing benefits coming from this effort.

Spirometry is the basis for diagnosing COPD but primary care providers, who first meet patients with respiratory symptoms, do not always have access [15], time or adequate training to use it [16]. In contrast to spirometry, our study showed that COPD-6™ can be reliably used in GP's offices with the results that are

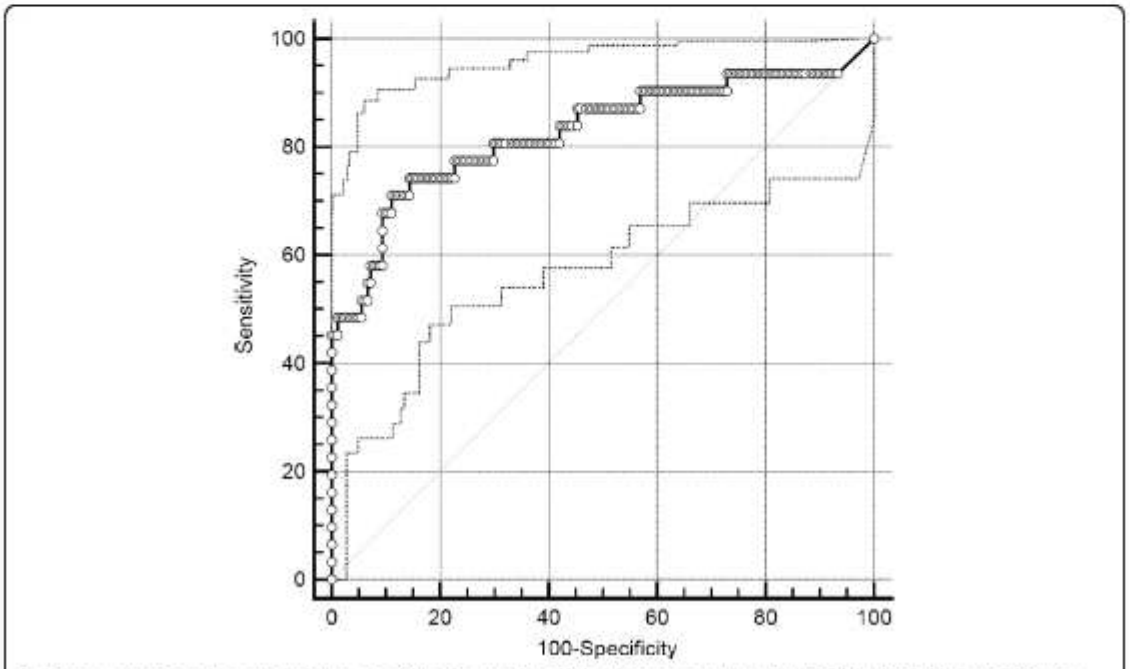

Fig. 2 ROC curve for the diagnosis of COPD using COPD-6™ at the GP's office. ROC curve plot (AUC 0.827, 95 % CI 0.769–0.875, *P* < 0.001) was based on FEV_1/FEV_6 measurements using COPD-6™ at the GP's office using COPD diagnosis made by pulmonologist at tertiary care hospital as criterion variable; dotted lines represent 95 % confidence intervals

Table 4 Methods comparison (Bland-Altman statistics) for lung function measurements performed in a GP's office and at lung function lab ($N = 227$)

		COPD-6™ at GP's office			
Lung function lab measurements			FEV_1 (L)	FEV_6 (L)	FEV_1/FEV_6 (%)
COPD-6™	FEV_1 (L)	Δ (95 % CI)	0.01 (−0.05 to 0.03)	NA	NA
	FEV_6 (L)	Δ (95 % CI)	NA	−0.17 (−0.24 to −0.12)*	NA
	FEV_1/FEV_6 (%)	Δ (95 % CI)	NA	NA	4.83 (3.71 to 6.35)*
Spirometry	FEV_1 (L)	Δ (95 % CI)	−0.12 (−0.15 to −0.09)*	NA	NA
	FVC (L)	Δ (95 % CI)	NA	−0.66 (−0.72 to −0.59)*	NA
	FEV_1/FVC (%)	Δ (95 % CI)	NA	NA	10.24 (9.32 to 11.26)*
Post-bronchodilator spirometry	FEV_1 (L)	Δ (95 % CI)	−0.15 (−0.19 to −0.12)*	NA	NA
	FVC (L)	Δ (95 % CI)	NA	−0.60 (−0.65 to −0.54)*	NA
	FEV_1/FVC (%)	Δ (95 % CI)	NA	NA	8.52 (7.57 to 9.47)*

GP general practitioner, *FEV_1* forced expiratory volume in 1 s, *FEV_6* forced expiratory volume in 6 s, *FVC* forced expiratory volume, *FVC* forced expiratory volume, *Δ* mean difference of the index test (COPD-6™ measurement at GP's office) from the reference (tertiary care measurement), *95 % CI* 95 % confidence interval *NA* not applicable
*$P < 0.001$

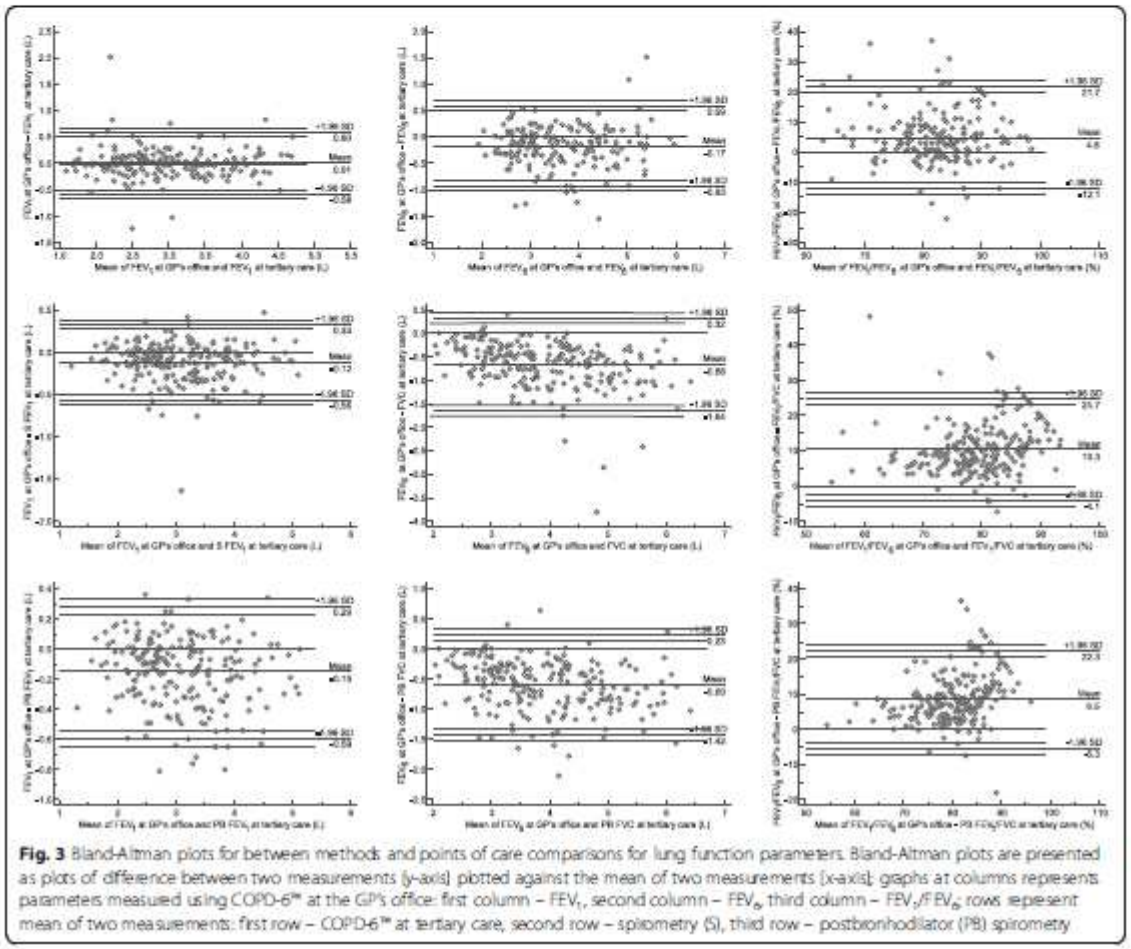

Fig. 3 Bland-Altman plots for between methods and points of care comparisons for lung function parameters. Bland-Altman plots are presented as plots of difference between two measurements (y-axis) plotted against the mean of two measurements (x-axis); graphs at columns represents parameters measured using COPD-6™ at the GP's office: first column – FEV_1, second column – FEV_6, third column – FEV_1/FEV_6; rows represent mean of two measurements: first row – COPD-6™ at tertiary care, second row – spirometry (S), third row – postbronhodilator (PB) spirometry

comparable to the measurements performed using the same device by the highly experienced and trained personnel.

Other studies evaluating the use of COPD-6™ differ in many aspects from our study. The population at risk was not strictly defined [7], the patients of older age were included thus increasing the probability of false positive results and inconclusive effects of screening with spirometry [12, 17, 18], and the results of FEV_1/FEV_6 measurements were not compared between GP's offices and lung function laboratory [19]. The results were mainly used as an advanced case finding technique and were not tested for diagnostic purposes. Different cut-off values for FEV_1/FEV_6 ratios were suggested with different sensitivity and specificity that didn't meet the criteria to establish the diagnosis of COPD [20, 21]. Based on the results of our study, the FEV_1/FEV_6 ratio <0.7 measured at GP's office has a specificity of 100 % with positive predictive value of 100 % indicating that a patient had a COPD and can be diagnosed in accordance to other diagnostic criteria with no further lung function testing needed. These results were good but significantly worse than spirometry. The same was the case for the FEV_1/FEV_6 ratio measured by COPD-6™ at a lung function lab at tertiary care hospitals using highly experienced staff. The reason behind it, lies in the lack of real-time visual control, present during spirometry, thus underestimating the real value of FEV_6 (a surrogate measure for FVC). This produced a systematic bias overestimating the real value of FEV_1/FEV_6 ratio. This points out that additional training should be provided to heath care personnel at GP's office to understand and recognize this possible measurement bias.

Otherwise, our exploratory analysis showed that FEV_1/FEV_6 ratio >0.85 measured at GP's office had a NPV of 95.74 % and was reasonable to conclude that a patient was not suffering from COPD and further testing was indicated to reveal the reasons for respiratory symptoms. A strategy of using this two cut-off values ($FEV_1/FEV_6 <$ 0.7 to diagnose and >0.85 to rule-out COPD) could increase the number of patients diagnosed at GP's offices and treated according to the current guidelines. A confirmatory study validating these thresholds should be done using these cut-off values as a pre-specified goals. If this thresholds are confirmed, only patients with the FEV_1/FEV_6 ratio between the 0.7 and 0.85 should be referred for further lung function testing and (sub)specialist evaluation. Our results were based on the study population from 40 to 65 years of age so in older populations recommendations from Global Lung Initiative (GLI) to use lower limit of normal (LLN) for FEV_1/FVC (different from a fixed criterion of <0.7) should be taken into account to prevent over diagnosing COPD in older population [12].

Diagnosing COPD is important because it was shown that undiagnosed patients with COPD have increased mortality [22], morbidity [5, 23] and decreased quality of life [24]. The treatment of such patients is delayed and the probability of quitting smoking is diminished [25]. For a decision to start the implementation of active case finding it is important to know the number of undiagnosed patients in a specific population [26]. There is evidence of different diagnostic accuracy of physician's established diagnosis for COPD in different countries and age groups [27]. Up till now, we didn't have a scientific data for our population. In our study, the number of undiagnosed COPD patients in a population at risk is approaching 20 % with almost half of them in advanced stages of the disease (8.4 %). These data are important for health authorities for decision making [28]. Our finding was on a lower end of the results from literature ranging from 20 % to more than 50 % of undiagnosed COPD patients in at risk population [29, 30]. This is probably the result of an overall education campaign started in our country as early as the year 2000 with the presentation of the COPD monograph (COPD guidelines developed by the Croatian Respiratory Society), and followed in subsequent years by the broad education campaign for both GPs and pulmonologists according to GOLD.

Based on the results of our study we could recommend the clinical algorithm (Fig. 4) for the triage of patient at risk for COPD (smokers/ex-smokers with >20 pack-years having chronic respiratory symptoms or comorbidities associated with COPD) using lung function testing with COPD-6™ at GP's office (for the age group of >70 cut-off value for the FEV_1/FEV_6 should be revised to LLN according to GLI recommendations [12]): (1) $FEV_1/FEV_6 < 0.7$ – treatment should be started according to the current guidelines in an uncomplicated patient or referred to specialist for further assessment if a patient is severe or has significant comorbidities; (2) $FEV_1/FEV_6 \geq 0.7$ and ≤ 0.85 – refer to pulmonologist for further consultation; (3) >0.85 should consider alternative diagnosis than COPD. Using such an algorithm in regular GP practice will allow GPs to make an early COPD diagnosis or a proper specialist referral thus producing more appropriate use of resources, cost savings and task shifting. These effects are based on much broader accessibility and lower price of services of general practice and limited resources on the secondary/tertiary care level considering the number of smokers in general population (up to 30 % of adults) and COPD patients (up to 10 % of adults). Substantial positive effects can be expected based on this broad accessibility regarding early diagnosis of COPD allowing early preventive interventions (quitting smoking) [25]. Although it can be supposed that an early intervention already in

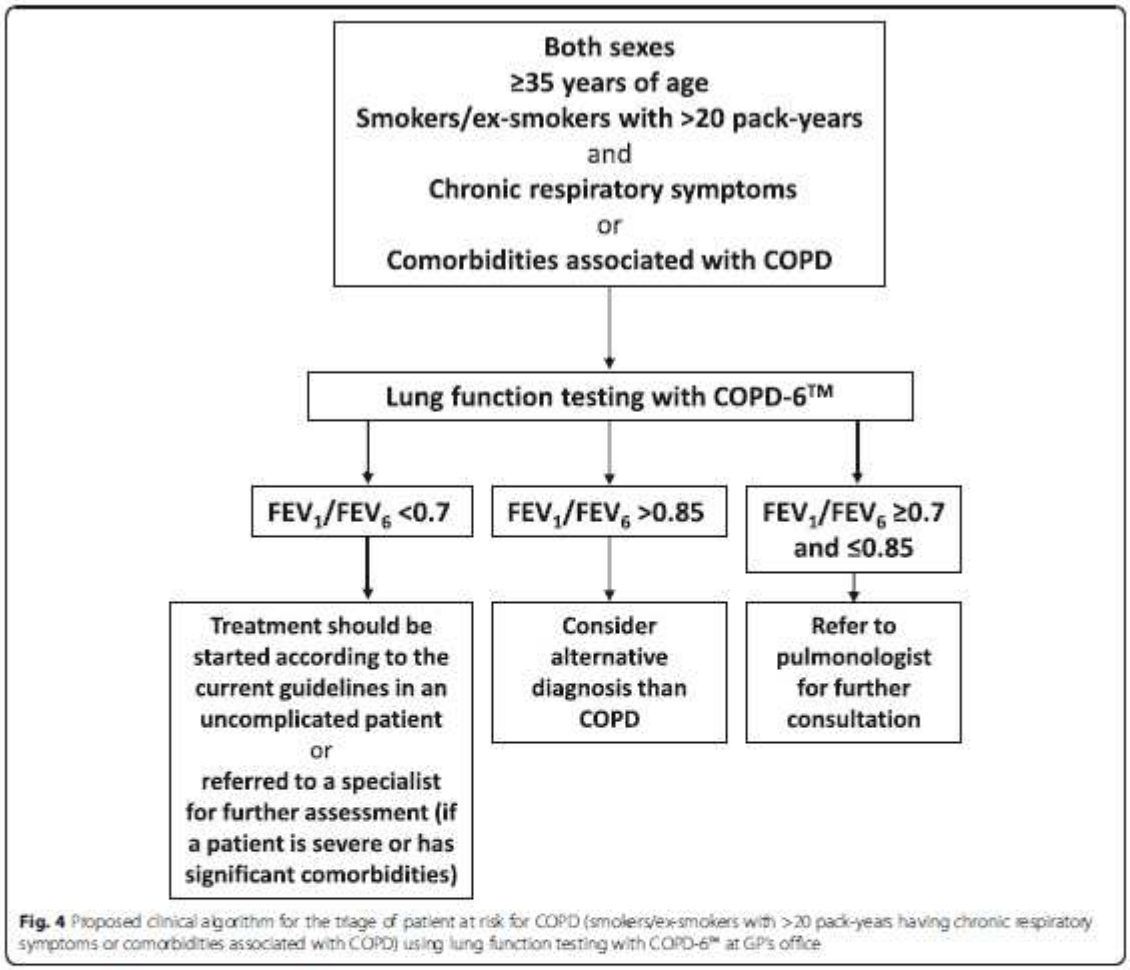

Fig. 4 Proposed clinical algorithm for the triage of patient at risk for COPD (smokers/ex-smokers with >20 pack-years having chronic respiratory symptoms or comorbidities associated with COPD) using lung function testing with COPD-6™ at GP's office

asymptomatic subjects with fixed airflow limitation could be beneficial there are no studies that confirm such a hypothesis and possible benefits but also no harm could be expected based on studies of therapeutic interventions in mild/moderate COPD [31].

The strengths of our study are based on the significant number of indicative COPD patients diagnosed through the diagnostic process ($n = 43$, 18.9 %), allowing us to properly evaluate the accuracy of the tested device. Also GPs were not extensively trained for the use of the tested device and were not aware to be part of the study thus allowing us to make conclusions that can be generalized to a regular real life clinical setup. Using a "gold standard" to make a COPD diagnosis on the referent level (tertiary care) as a reference standard and evaluation of lung function measurements conducted by GPs against spirometry conducted by experienced staff provides us with an objective evaluation of the tested device and GPs performance. The possible weaknesses of our study are based on recruitment process that may not represent the actual general practice population thus possibly diminishing generalizability of our results, although our analysis showed that there was no clustering present regarding the characteristics of patients recruited by different GPs and the proportion of undiagnosed COPD patients was comparable to other studies. Also the age range of our study participants (40–65 years of age) does not allow us to generalize our results outside this age range thus leaving the most questionable population regarding the diagnostic criteria out of our focus (>70 years of age). A bias could be present because there was no formal panel diagnosis of COPD done, but the diagnosis was done using the harmonized criteria by experienced pulmonologists (all pulmonologist making a diagnosis were acting as trainers for more than 10 years for GOLD initiative in Croatia). So for our data

to become more generalizable our research needs to be conducted in a broader population (age 35–80 years, >10 pack-years) using also two threshold values that were found out in our exploratory analyses ($FEV_1/FEV_6 < 0.7$ and >0.85).

Conclusions

Almost one fifth (18.9 %) of undiagnosed patients with COPD in a population at risk (smokers/ex-smokers) in our study points to the fact that active case finding should be instituted in such a population. Based on the results of our study lung function testing with COPD-6™ can in a significant part substitute spirometry in cases where it is not readily available to the patient/physician. Results of lung function testing with COPD-6™ performed at GP's offices and in lung function laboratory were comparable, so there is a possibility to establish the diagnosis of COPD and start adequate treatment in a GP's office in a substantial number of patients at risk. This could be based on two cut-of values for FEV_1/FEV_6, with the ratio <0.7 establishing and >0.85 excluding the diagnosis of COPD. Before the implementation in practice, the diagnostic criteria should be checked for a specific population. Such approach could lead to a better diagnostic yield of COPD in everyday practice diminishing the number of underdiagnosed and over-diagnosed patients.

Acknowledgements
The authors wish to thank the consultant Prof. Peter MA Calverley for valuable advice for the study design. The authors would also like to thank the following researchers who helped in the recruitment of participants and the data collection: Vjekoslava Ameršek, MD; Ines Balint, MD; Merim Bestrov, MD; Mirjana Bestrov, MD; Ivana Boban, MD; Ljiljana Bulat Kardum, MD, PhD; Ines Diminić Lisica, MD, PhD; Albina Dumić, MD, PhD; Ljiljana Ismić, MD; Tajana Jakušić Glunčić, MD; Renata Giguzić, MD; Monika Jeđud, MD; Ervo Ljubičić, MD; Tina Lukić, MD; Ljiljana Lulić-Kasapetić, MD; Suzana Maltar-Delija, MD; Dubravka Margaretić, MD; Davorka Martinković, MD; Zdenka Meštrović, MD; Nataša Meduljaš-Đuić, MD; Jasna Nagyszombaty-Šarić, MD; Darja Nelken-Bestvina, MD; Sanja Popović Grle, MD, PhD; Sanda Pribić, MD; Jadranka Radman, MD; Rosanda Rosandić Plasevoli, MD; Alen Stojanović, MD; Karla Tudja, MD; Neven Tudorić, MD, PhD; Bojana Vukanjac, MD; Vanja Vuli, MD.

Funding
The study was funded by the unrestricted grant from GlaxoSmithKline (*GSK eTrack number*: CRT114338).

Availability of data and materials
As this is a clinical dataset, data is not publicly available because that would breach the ethics committee approval (issued in 2010) and informed consent that was signed by the participants of this trial (signed in 2010–2012).

Authors' contributions
All authors contributed to the original ideas and writing of this paper. Authorship of ML and ZV should be evaluated as equally contributing first authors. ML participated in study design and coordination, data acquisition and drafting the manuscript. ZV participated in conceiving the study, its design and coordination and drafting the manuscript. IG participated in study design and coordination, data acquisition and helped to draft the manuscript. SI. participated in study coordination, data acquisition and helped to draft the manuscript. DP participated in conceiving the study, its design and coordination, data acquisition, performed the statistical analysis and drafted the manuscript. All authors read and approved the final manuscript.

Competing interests
The study was in part funded by the unrestricted grant from GlaxoSmithKline (*GSK eTrack number*: CRT114338). Prof. Davor Plavec and Dr. Žarko Vrbica as principal investigators and Children's Hospital Srebrnjak have for the purpose of investigator initiated study MARKO (ClinicalTrials.gov Identifier NCT01550679) received an unrestricted grant from GlaxoSmithKline. GlaxoSmithKline has not influenced the study design or study protocol, has no ownership rights over data gathered by the study and has no influence on this and further publications coming from data gathered through this study. The authors declare that they have no competing interests in relation to this manuscript.

Consent for publication
Not applicable.

Ethics approval and consent to participate
The study was approved by the Children's Hospital Srebrnjak Ethics Committee (No. 01-2191/1-2010) and conducted according to the Declaration of Helsinki and other relevant international and national laws. The patients were approached by their GPs during any (unrelated to respiratory problems) visit to their office if they were smokers or ex-smokers of the predefined age group for the study together with the prescreening for inclusion/exclusion criteria using a structured interview. Eligible patients were given the Informed consent document with enough time to read it and to discuss any relevant issues regarding the study before they signed the written consent. They were informed about the prospective nature of the study and their right to withdraw their consent and claim the withdrawal of all gathered data and destroying all biological samples at any time without any explanation, obligation or consequence from their side. All participants signed the written consent before starting any procedure for the study.

Author details
[1]Department of Pulmonology, University Hospital Center Osijek, Josipa Huttlera 4, Osijek, Croatia. [2]Faculty of Medicine, J.J. Strossmayer University of Osijek, Ulica cara Hadrijana 10E, Osijek, Croatia. [3]Department of Pulmonology an Immunology, General Hospital Dubrovnik, Dr. Roka Mišetića 2, Dubrovnik, Croatia. [4]University of Dubrovnik, Branitelja Dubrovnika 29, Dubrovnik, Croatia. [5]Department of Pulmonology, University Hospital Center Split, Spinčićeva 1, Split, Croatia. [6]Research Department, Children's Hospital Srebrnjak, Srebrnjak 100, Zagreb, Croatia.

Received: 3 May 2016 Accepted: 16 August 2016
Published online: 19 August 2016

References

1. Global Initiative for Chronic Obstructive Lung Disease (GOLD). 2015. Available from: URL: http://www.goldcopd.org/. Accessed 6 June 2016.
2. Bednarek M, Maciejewski J, Wozniak M, Kuca P, Zielinski J. Prevalence, severity and underdiagnosis of COPD in the primary care setting. Thorax. 2008;63:402–7.
3. Jones RC, Price D, Ryan D, Sims EJ, von Ziegenweidt J, Mascarenhas L, Respiratory Effectiveness Group, et al. Opportunities to diagnose chronic obstructive pulmonary disease in routine care in the UK: a retrospective study of a clinical cohort. Lancet Respir Med. 2014;2:267–76.
4. Bruscas Alijarde MJ, Naberan TK, Lamban Sanchez MT, Bello Dronda S. ARAPOC Study: Prevalence of respiratory symptoms and chronic obstructive pulmonary disease in the general population. Aten Primaria. 2014;1091:50212–9.
5. Roche N, Gaillat J, Garre M, Meunier JP, Lemaire N, Bendjenana H. Acute respiratory illness as a trigger for detecting chronic bronchitis in adults at risk of COPD: a primary care survey. Prim Care Respir J. 2010;19:371–7.
6. Welte T, Vogelmeier C, Papi A. COPD: early diagnosis and treatment to slow disease progression. Int J Clin Pract. 2015;69:336–49.
7. Yawn BP, Duvall K, Peabody J, Albers F, Iqbal A, Paden H, et al. The impact of screening tools on diagnosis of chronic obstructive pulmonary disease in primary care. Am J Prev Med. 2014;47:563–75.
8. Leuppi JD, Miedinger D, Chhajed PN, Buess C, Schafroth S, Bucher HC, Tamm M. Quality of spirometry in primary care for case finding of airway obstruction in smokers. Respiration. 2010;79:469–74.
9. Koefoed M, Sondergaard J, dePont Christensen R, Jarbol DE. General practice variation in spirometry testing among patients receiving first-time

prescriptions for medication targeting obstructive lung disease in Denmark: a population-based observational study. BMC Fam Pract. 2013;14:113.
10. Zwar NA, Marks GB, Hermiz O, Middleton S, Comino EJ, Hasan I, et al. Predictors of accuracy of diagnosis of chronic obstructive pulmonary disease in general practice. Med J Aust. 2011;195:168–71.
11. Miller MR, Levy ML. Chronic obstructive pulmonary disease: missed diagnosis versus misdiagnosis. BMJ. 2015;351:h3021–6.
12. Stanojevic S, Quanjer P, Miller RM, Stocks J. The Global Lung Function Initiative: dispelling some myths of lung function test interpretation. Breathe. 2013;9:463–74.
13. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. ATS/ERS Task Force: ATS/ERS Task Force: Standardisation of spirometry. Eur Respir J. 2005;26:319–38.
14. Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC. Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. Eur Respir J. 1993;6 Suppl 16:5–40.
15. Joo MJ, Lee TA, Weiss KB. Geographic variation of spirometry use in newly diagnosed COPD. Chest. 2008;134:38–45.
16. Walters JA, Hansen EC, Johns DP, Blizzard EL, Walters EH, Wood-Baker R. A mixed methods study to compare models of spirometry delivery in primary care for patients at risk of Chronic Obstructive Pulmonary Disease. Thorax. 2008;63:408–14.
17. Ching SM, Pang YK, Price D, Cheong AT, Lee PY, Imm I, et al. Detection of airflow limitation using a handheld spirometer in a primary care setting. Respirology. 2014;19:689–93.
18. Bertens LC, Reitsma JB, van Mourik Y, Lammers JW, Moons KG, Hoes AW, et al. COPD detected with screening: impact on patient management and prognosis. Eur Respir J. 2014;44:1571–8.
19. Thorn J, Tilling B, Lisspers K, Jörgensen L, Sterling A, Stratelis G. Improved prediction of COPD in at-risk patients using lung function pre-screening in primary care: a real-life study and cost-effectiveness analysis. Prim Care Respir J. 2012;21:159–66.
20. Sichletidis L, Spyratos D, Papaioannou M, Chloros D, Tsiotsios A, Tsagaraki V, Haidich AB. A combination of the IPAG questionnaire and PiKo-6 flow meter is a valuable screening tool for COPD in the primary care setting. Prim Care Respir J. 2011;20:184–9.
21. Frith P, Crockett A, Beilby J, Marshall D, Attewell R, Ratnarajan A, Gavagna G. Simplified COPD screening: validation of the PiKo-6 in primary care. Prim Care Respir J. 2011;20:190–8.
22. Sin DD, Wu L, Man SF. The relationship between reduced lung function and cardiovascular mortality: a population-based study and a systematic review of the literature. Chest. 2005;127:1952–9.
23. Young RP, Hopkins RJ, Christmas T, Black PN, Metcalf P, Gamble GD. COPD prevalence is increased in lung cancer, independent of age, sex and smoking history. Eur Respir J. 2009;34:380–6.
24. Jordan RE, Lam KB, Cheng KK, Miller MR, Marsh JL, Ayres JG, et al. Case finding for chronic obstructive pulmonary disease: a model for optimising a targeted approach. Thorax. 2010;65:492–8.
25. Parkes G, Greenhalgh T, Griffin M, Dent R. Effect on smoking quit rate of telling patients their lung age: the Step2quit randomised controlled trial. BMJ. 2008;336:598–600.
26. Gruffydd-Jones K. A national strategy for the management of chronic obstructive pulmonary disease (COPD) in England: aiming to improve the quality of care for patients. Prim Care Respir J. 2008;17 Suppl 1:S1–8.
27. Vajer P, Szelvari A, Voros K, Torzsa P, Fory A, Dunai K, et al. Comparative analysis of diagnostic probability estimates of some common diagnoses among family doctors, medical residents, and medical students reveals negative correlation between age and estimate of chronic obstructive pulmonary disease (COPD). Med Sci Monit. 2010;16:CR109–15.
28. Punekar YS, Wurst K, Shukla A. Resource Use and Costs up to Two Years Post Diagnosis Among Newly Diagnosed COPD Patients in the UK Primary Care Setting: A Retrospective Cohort Study. COPD. 2015;12:267–75.
29. Mannino DM, Gagnon RC, Petty TL, Lydick E. Obstructive lung disease and low lung function in adults in the United States: data from the National Health and Nutrition Examination Survey, 1988–1994. Arch Intern Med. 2000;160:1683–9.
30. Jithoo A, Enright PL, Burney P, Buist AS, Bateman ED, Tan WC, BOLD Collaborative Research Group, et al. Case-finding options for COPD: Results from the BOLD Study. Eur Respir J. 2013;41:548–55.
31. US Preventive Services Task Force (USPSTF), Siu AL, Bibbins-Domingo K, Grossman DC, Davidson KW, Epling Jr JW, Garcia FA, et al. Screening for Chronic Obstructive Pulmonary Disease: US Preventive Services Task Force Recommendation Statement. JAMA. 2016;315:1372–7.

3.4. Exhaled Breath Temperature as a Novel Marker of Future Development of COPD: Results of a Follow-Up Study in Smokers.

COPD: JOURNAL OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE
2016, VOL. 0, NO. 0, 1–9
http://dx.doi.org/10.3109/15412555.2016.1164129

Taylor & Francis
Taylor & Francis Group

Exhaled Breath Temperature as a Novel Marker of Future Development of COPD: Results of a Follow-Up Study in Smokers

Marina Labor[a,e], Žarko Vrbica[b,c], Ivan Gudelj[d], Slavica Labor[a,e], Iva Jurič[f], and Davor Plavec[e,g]

[a]Department of Pulmonology, University Hospital Center Osijek, Osijek, Croatia; [b]Department of Pulmonology and Immunology, General Hospital Dubrovnik, Dubrovnik, Croatia; [c]University of Dubrovnik, Dubrovnik, Croatia; [d]Department of Pulmonology, University Hospital Center Split, Split, Croatia; [e]Faculty of Medicine, J.J. Strossmayer University of Osijek, Osijek, Croatia; [f]Department of Cardiology, University Hospital Center Osijek, Osijek, Croatia; [g]Research Department, Children's Hospital Srebrnjak, Zagreb, Croatia.

ABSTRACT

Although only less than one-third of smokers develop COPD, early marker(s) of COPD development are lacking. The aim of this research was to assess the ability of an average equilibrium exhaled breath temperature (EBT) in identifying susceptibility to cigarette smoke so as to predict COPD development in smokers at risk. The study was a part of a multicenter prospective cohort study in current smokers (N = 140, both sexes, 40–65 years, ≥20 pack-years) with no prior diagnosis of COPD. Diagnostic workup includes history, physical, quality of life, hematology and highly sensitive CRP, EBT before and after smoking a cigarette, lung function with bronchodilator test, and 6-minute walk test. Patients without a diagnosis of COPD and in GOLD 1 stage at initial assessment were reassessed after 2 years. COPD was additionally diagnosed based on lower level of normal (LLN) lung function criteria. Utility of EBT for disease progression was analyzed using receiver operator curve (ROC) and logistic regression analyses. Change in EBT after smoking a cigarette at initial visit (ΔEBT) was significantly predictive for disease progression (newly diagnosed COPD; newly diagnosed COPD + severity progression) after 2 years ($p < 0.05$ for both). ΔEBT had an AUC of 0.859 ($p = 0.011$) with sensitivity of 66.7% and specificity of 98.1% for newly diagnosed COPD using LLN criteria. We conclude that EBT shows potential for predicting the future development of COPD in current smokers. This was best seen using LLN to diagnose COPD, adding further evidence to question the use of GOLD criteria for diagnosing COPD.

KEYWORDS

Biological markers; chronic obstructive pulmonary disease (COPD); cigarette smoking; cohort study; disease susceptibility; exhaled breath temperature; trial registration

Introduction

Although we understand that cigarette smoke is a major environmental risk factor involved in the development of chronic obstructive pulmonary disease (COPD), we still have not unveiled the epigenetic regulatory mechanisms of oxidative genes involved in its pathogenesis (1, 2). Only less than one-third of smokers develop symptomatic disorder (primarily COPD) during lifetime, suggesting that there is an individual genetic or epigenetic background, making them susceptible to cigarette smoke (2). Progressive nature of COPD inflicts significant disability and later also an early mortality, thus producing serious public health and economic impact for the health system and economy in general. Therefore, stopping or slowing down the progression of the disease is a main therapeutic goal (3). Therapeutic interventions in COPD patients have been shown to have significantly larger impact if they were started earlier in the course of the disease (4–6). An early diagnosis should allow an early intervention, thereby preventing the progression of COPD, alleviating the symptoms, improving the tolerance of exertion and general wellbeing, preventing complications and co-morbidities and early mortality (7). Finding a marker of susceptibility to cigarette smoke that can predict the future development of COPD, before the significant end-organ damage, is still an unmet need in the management of COPD. Despite the significant efforts of the scientific community during the last decade in trying to find such markers for COPD, we are still far from that goal. MARKO project (https://clinicaltrials.gov/ct2/show/NCT01550679) was started with this aim by recruiting patients with no previous diagnosis of COPD, but at risk for future development of disease, having significant age and cigarette smoke exposure (smokers/ex-smokers aged 40–65 years with smoking history of ≥20 pack-years).

There are significant pathohistological changes of the airways associated with the duration and progression of COPD, and depending on the phenotype, a significant decrease in bronchial vascularity can be found (8–11). On the other hand, it has been shown that chronic inflammation of the airways induces vascular proliferation, as also seen in some COPD phenotypes (8). As a consequence of inflammation and remodeling/destruction of the airways/parenchyma, changes in the bronchial blood flow affect the heat and water vapor exchange in the airways, thereby affecting the exhaled breath temperature (EBT). Changes, such as the growth of submucosal capillary network, medial and intimal hyperplasia and/or exudation, can directly influence the airway wall thickness, thus influencing the heat exchange (12). It has been shown that EBT can be used as an individual measure of fluctuating changes in the balance of these processes (13). Recently, EBT has been proposed to reflect airways

CONTACT Davor Plavec plavec@bolnica-srebrnjak.hr Head of Research Department, Children's Hospital Srebrnjak, Srebrnjak 100, Zagreb, Croatia.
Marina Labor and Žarko Vrbica are equally contributing first authors.

inflammation, as a positive relationship was observed between EBT, bronchial blood flow and exhaled nitric oxide in asthmatic patients (14, 15). Also, it has been suggested as a new method to detect and monitor pathological processes in asthma, COPD and lung cancer (16).

Based on the pathophysiological changes in COPD and also previous studies showing significant change in EBT in patients with inflammatory respiratory disorders (including COPD), this study aimed at testing if measuring EBT in current smokers at COPD risk can show susceptibility to cigarette smoke and be predictive for future development of COPD and/or disease progression.

Methods

Study framework

This study is a part of the broader research project (Early detection of COPD patients in GOLD 0 (smokers) population – MARKO project). The whole protocol of the MARKO project can be found at https://clinicaltrials.gov/ct2/show/NCT01550679. In short, the MARKO project is a prospective, observational, non-interventional cohort study of patients (both sexes) at risk for the development of COPD (smokers/ex-smokers with a smoking history of ≥20 pack-years), without a previous diagnosis of COPD. The project was approved by Local Ethics Committees and carried out in accordance with the Declaration of Helsinki, GCP, and all relevant international and national legislation. The patients were approached by their general practitioners (GPs) during any (unrelated to respiratory problems) visit to GP's office if they were smokers or ex-smokers of the predefined age group for the study, together with the prescreening for inclusion/exclusion criteria using a structured interview. Eligible patients were given the informed consent document with enough time to read it and to discuss any relevant issues regarding the study before consenting. All participants signed a written consent before entering the study and before any procedure was performed.

Subjects

For this study, 146 consecutive patients from 26 GP offices were recruited into the study by their GPs based on the inclusion criteria: written consent; current smokers (because they had to smoke a cigarette as a part of a study protocol) of both sexes aged 40–65 years with a smoking history of at least 20 pack-years (calculated as a number of cigarettes smoked per day multiplied by the number of years of smoking divided by 20); and no previous diagnosis of COPD. Exclusion criteria were: any clinically relevant chronic disease (cardiovascular, cerebrovascular, diabetes, hepatitis, nephropathy, chronic dialysis, systemic disorder, cancer) significantly affecting QoL; ongoing immunosuppressive therapy; preceding acute respiratory disease 4 weeks before inclusion; hospitalization for any reason during past 3 months; myocardial infarction (MI), cerebrovascular infarction (CVI) or transient ischemic attack (TIA) during past 6 months; diagnosis of asthma; and an inability to perform the diagnostic protocol. Six patients were excluded from the analyses because their baseline EBT (EBTb) values were extremely low (<25°C), suggesting possible artifacts in the measurement so the data from 140 subjects were analyzed.

Study workup

After the structured history and examination at the GP's office and lung function testing with COPD-6 (4000 COPD-6™ Respiratory Monitor, Vitalograph Ltd., Buckingham, UK), patients were referred to the tertiary care hospital having a designated team consisting of a pulmonologist, research nurse and lung function laboratory technician. A structured diagnostic workup comprising QoL questionnaires, structured history and physical examination, EBT before (EBTb) and after smoking cigarette (EBTc), lung function testing with bronchodilator (salbutamol), laboratory [hematology and high-sensitivity C-reactive protein (hs-CRP)], functional status using 6-minute walk test (6MWT), ending with the assessment for diagnosis and severity of COPD according to GOLD (17) was performed. Patients with no COPD and with COPD GOLD 1 stage [postbronchodilator (PB) ratio of forced expiratory volume in 1 second and forced vital capacity (FEV_1/FVC) < 0.7 and FEV_1 ≥ 80% predicted] after initial assessment were included in the follow-up in accordance with a predefined study protocol. These patients were reassessed by the same pulmonologist after 2 years for diagnosis and progression of COPD. In the case of acute respiratory disease, preceding or at the time of a follow-up visit, subjects were rescheduled to present at least 4 weeks after the resolution of this acute episode.

Outcomes

Primary outcome was to assess the predictive potential of the change in EBT after smoking a cigarette (ΔEBT) using three different outcome measures for the progression of disease after 2 years of follow-up in patients at risk (active smokers with smoking history of ≥20 pack-years without COPD or with a COPD in GOLD 1 stage after baseline assessment): (1) newly diagnosed COPD (ND COPD); (2) disease progression (DP; ND COPD + progression to a higher severity stage); and (3) higher rate of loss of lung function (LoLF).

Secondary outcomes were to assess the predictive potential of (1) baseline EBT (EBTb) and (2) EBT after smoking cigarette (EBTc) using three outcome measures of DP after 2 years of follow-up in patients at risk (as for the primary outcome).

Exploratory outcome was to assess the associations of outcome measures with EBT and patients' baseline characteristics [age, sex, smoking status, comorbidities, lung function, functional exercise capacity, laboratory parameters, and health-related QoL (HRQoL)].

Outcome measures (ND COPD, DP) were defined using the definition of COPD and COPD severity criteria in GOLD (17); (1) ND COPD – subjects with chronic respiratory symptoms (dyspnea, cough or sputum), a history of exposure to risk factors and a persistent airflow limitation (PB FEV_1/FVC < 0.7) at a control visit after 2 years if they were not diagnosed according to the same criteria at baseline visit; (2) DP – subjects with ND COPD + subjects that progressed from GOLD 1 severity stage at baseline visit to GOLD 2 or higher severity stage based on the % predicted value of FEV_1 at a control visit after 2 years. To assess if the progression of airflow limitation (PB FEV_1/FVC

going below 0.7 or PB FEV_1 going below 50% predicted after 2 years) was just the function of age and not the disease, more stringent criteria for airflow limitation using a lower level of normal (LLN) proposed by Global Lung Initiative (GLI) and American Thoracic Society/European Respiratory Society (ATS/ERS) were also used for both outcome measures (18, 19). For ND COPD, the criterion was PB FEV_1/FVC going below LLN after 2 years, and for DP it was PB FEV_1/FVC going below LLN or PB FEV_1 going below LLN (if FEV_1/FVC was below LLN at a baseline visit) after 2 years.

Outcome measure LoLF was defined not only as a loss of PB $FEV_1 > 70$ mL/year but also as a loss in FEV_1 defined as > 1 standardized residual (SR) units after 2 years of follow-up to correct the flaw coming from using % predicted values that retain sex, age and height bias (18, 20).

Health-related quality of life

HRQoL was assessed using two questionnaires; St. George Respiratory Questionnaire (SGRQ) and COPD Assessment Test (CAT). The SGRQ is a standardized self-administered airways disease-specific questionnaire divided into three subscales: symptoms (8 items), activity (16 items), and impacts (26 items). SGRQ scores were calculated using score calculation algorithms and missing data imputation (if total number of missing items was ≤ 10) using the Excel® SGRQ calculator. For each subscale and for the overall questionnaire, the scores range from zero (no impairment) to 100 (maximum impairment) (21). The CAT is a validated, short (8-item) and simple patient completed questionnaire, with good discriminant properties, developed for use in routine clinical practice to measure the health status of patients with COPD. Every item has a scale of 0–5 with scoring range from zero (no impairment) to 40 (maximum impairment) (22). Both QoL questionnaires were self-completed by patients before any other procedure was done.

Lung function

Spirometry was done by computerized pneumotachographs (Jaeger®, CareFusion, CA, USA) using the same procedure at all clinical sites (lung function labs at tertiary hospitals), harmonizing the procedure according to ATS/ERS guidelines (23). The best of three technically satisfactory efforts was recorded. Bronchodilator test was done with the repeated spirometry 20 minutes after the inhalation of 400 mcg of salbutamol using the inhalation chamber. Spirometric indexes [FVC, FEV_1, FEV_1/FVC, peak expiratory flow (PEF)] at baseline and after bronchodilator (PB) were recorded as absolute values and as percentage of predicted values according to Quanjer (18). The values were also expressed as SRs and an LLN was calculated for FEV_1 and FEV_1/FVC according to GLI (18, 24).

Exhaled breath temperature

EBT was measured using X-Halo® device (Delmedica Investments, Singapore) according to the previously validated method (25, 26). The device does not measure an instantaneous EBT but an average equilibrium temperature. Measurements using this device and type of breathing, compared to other methods, did not show the influence of environmental factors and lung volume on the measured EBT (14, 27). Patients were requested to inhale freely through their nose and to exhale through mouth into the device at a rate and depth typical of their normal tidal breathing pattern using a one-way valve (preventing the rebreathing through device) and were closely monitored by the staff during the measurement. The measurement was continued until the software of the instrument indicated that the measured value was stable, thus fulfilling the criteria of a previously described mathematical model; the instrument processed an incremental temperature curve in relation to the initial temperature of the air in the thermal chamber of the instrument and was able to capture the achievement of the temperature plateau within an error of $< 2\%$. The reproducibility of the EBT measurements performed by X-Halo® had been previously demonstrated to have an intraclass correlation coefficient of 0.99 (14). The tests were carried out at a room temperature of 19–25°C, and at relative humidity of 30–60% in the lung function lab where the atmosphere is controlled, measured and logged. If the measured EBT was out of the expected range (30–35°C) the measurement was repeated after the temperature of X-Halo® device decreased to room temperature. In such a case, the measurement was repeated (no more than 3 times) until 2 repeatable measurements were obtained (difference < 0.1°C) and an average of the two was recorded. Patients who had an EBT < 25°C, even after repeated measurements, were excluded from analysis ($n = 6$). EBT was measured twice on the same occasion (during the initial visit); (1) baseline measurement before any other procedure (lung function, bronchodilator test, 6MWT) at least 1 h after smoking cigarette (EBTb) and (2) 15 minutes after smoking cigarette (EBTc) and recorded with a precision of 1/100 of 1°C. Subjects also avoided food, beverages and medicine consumption at least 2 hour before measurement. No other procedure except cigarette smoking was performed between two EBT measurements. The change in EBT after smoking cigarette (ΔEBT) was calculated as the absolute difference between EBTc and EBTb ($\Delta EBT = EBTc - EBTb$).

Other procedures

Laboratory measurements were conducted in local clinical laboratories and included complete blood cell count, white blood cells differential count, hematocrit, hemoglobin and hs-CRP.

Functional exercise capacity was assessed using 6MWT according to the ATS guidelines and expressed as walked distance in meters and as % predicted according to Trooster et al. (28, 29).

Data analysis

Data analysis was done using STATISTICA version 12 (StatSoft, Inc., OK, USA) and MedCalc Statistical Software version 15.8 (MedCalc Software bvba, Ostend, Belgium; https://www.medcalc.org; 2015). Categorical data was presented as absolute and relative (%) numbers. Continuous variables were presented as mean and standard deviations (SD). Categorical data was

Table 1. Characteristics of patients at initial visit according to sex (N = 140).

Variable*	All (N = 140)	Women (n = 76)	Men (n = 64)	Statistics†
Age (years)	53.0 ± 6.4	53.5 ± 5.9	52.4 ± 6.9	$t = 1.042$; $p = 0.299$
BMI (kg/m²)	26.58 ± 3.97	25.89 ± 3.97	27.40 ± 3.83	**$t = 2.274$; $p = 0.025$**
Pack-years	38.5 ± 17.9	33.2 ± 15.0	44.7 ± 19.3	**$t = 3.970$; $p < 0.001$**
FVC (%Pred, SR)	111.38 ± 15.41, 0.84 ± 1.14	113.35 ± 16.54, 0.96 ± 1.18	108.93 ± 13.63, 0.70 ± 1.07	$t = 1.870$; $p = 0.064$, $t = 1.370$; $p = 0.173$
FEV_1 (%Pred, SR)	100.42 ± 13.60, 0.03 ± 0.96	100.54 ± 14.12, 0.04 ± 0.98	100.27 ± 13.04, 0.03 ± 0.95	$t = 0.116$; $p = 0.908$, $t = 0.061$; $p = 0.951$
FEV_1/FVC (ratio, SR)	0.749 ± 0.064, −0.51 ± 0.94	0.759 ± 0.060, −0.47 ± 0.97	0.738 ± 0.066, −0.55 ± 0.91	$t = 1.918$; $p = 0.057$, $t = 0.492$; $p = 0.623$
PB FVC (%Pred, SR)	109.94 ± 14.51, 0.74 ± 1.07	111.92 ± 15.03, 0.83 ± 1.07	107.54 ± 13.60, 0.63 ± 1.06	$t = 1.773$; $p = 0.078$, $t = 1.113$; $p = 0.268$
PB FEV_1 (%Pred, SR)	101.24 ± 13.96, 0.09 ± 0.97	101.12 ± 14.38, 0.08 ± 0.98	101.38 ± 13.54, 0.10 ± 0.97	$t = 0.104$; $p = 0.918$, $t = 0.128$; $p = 0.898$
PB FEV_1/FVC (ratio, SR)	0.763 ± 0.062, −0.28 ± 0.91	0.771 ± 0.054, −0.25 ± 0.88	0.753 ± 0.068, −0.32 ± 0.95	$t = 1.661$; $p = 0.099$, $t = 0.398$; $p = 0.691$
Δ PB FEV_1 (%)	1.28 ± 4.99	1.04 ± 3.59	1.54 ± 6.19	$t = 0.477$; $p = 0.635$
6MWT (%Pred)	64.25 ± 13.49	67.44 ± 11.31	60.39 ± 14.95	**$t = 2.878$; $p = 0.005$**
EBTb (°C)	33.33 ± 2.30	32.94 ± 2.67	33.80 ± 1.67	$Z = 1.108$; $p = 0.268$
EBTc (°C)	33.22 ± 2.10	32.79 ± 2.56	33.81 ± 0.96	$Z = 1.207$; $p = 0.227$
ΔEBT (°C)	−0.07 ± 1.42	−0.13 ± 1.45	0.01 ± 1.40	$Z = 0.755$; $p = 0.755$

Legend: *all values are presented as mean ± SD; †statistical significance was tested using Student's *t*-test (*t* value) or Mann-Whitney U-test (*Z* value); BMI – body mass index (calculated as weight in kg divided by the squared height in m); FVC – forced vital capacity as % predicted (%Pred) or as standardized residuals (SR); FEV_1 – forced expiratory volume in 1 second as % predicted (%Pred) or as standardized residuals (SR); FEV_1/FVC as ratio or as standardized residuals (SR); PB – postbronchodilator value; Δ – % change after bronchodilator; 6MWT – 6-minute walk test as % predicted (%Pred); EBT – exhaled breath temperature; EBTb – baseline EBT; EBTc – EBT after smoking cigarette; ΔEBT – change in EBT after smoking cigarette.

compared between subgroups using chi-square test and continuous variables using Student's *t*-test or Mann-Whitney U-test, analysis of variance (ANOVA) or Kruskal-Wallis ANOVA after assessing the criteria for the use of parametric tests. Utility of EBT (EBTb, EBTc and ΔEBT) for DP was analyzed using receiver operator curve (ROC) analysis, and data was presented as AUC (with 95% confidence intervals, CIs) together with associated criterion, sensitivity, specificity and positive (PPV) and negative predictive (NPV) values. Association of outcome measures with EBT and baseline characteristics was tested using logistic regression analysis using stepwise approach. $P < 0.05$ was considered statistically significant for all analyses.

Results

Description of the cohort

One hundred and forty patients (76 women) aged 40–65 years at entry were included in this cohort study. Women had a mean (SD) age of 53.5 (5.9) years and men had a mean age of 52.4 (6.9) years ($p = 0.419$) with a mean smoking history of 33.2 (15.0) years and 44.7 (19.3) pack-years—men having a significantly greater cumulative exposure ($t = 3.970$; $p < 0.001$). The only functional index significantly different between sexes was 6MWT expressed as the % of predicted values significantly lower in men (mean ± SD; 60.39 ± 14.95% vs. 67.44 ± 11.31%, $t = 2.878$; $p = 0.005$), and no significant difference was found for lung function or EBT ($p > 0.05$ for all, Table 1). No significant association was found between EBTb and ΔEBT with age (Spearman R = −0.143 and −0.069, $p = 0.092$ and $p = 0.448$, respectively; Figures 1 and 2) and with lung function indexes (baseline and postbronchodilator) in a univariate analysis ($p > 0.05$ for all). Seventy-one (50.7%) patients had 1–3 co-morbidities and 62 (44.3%) were using 1–4 medications as a chronic treatment. After the initial pulmonologist assessment, 81 (57.9%) patients could be classified as symptomatic smokers (FEV_1/FVC ≥ 0.70) and 22 (15.7%) as COPD GOLD 1 stage (Table 2).

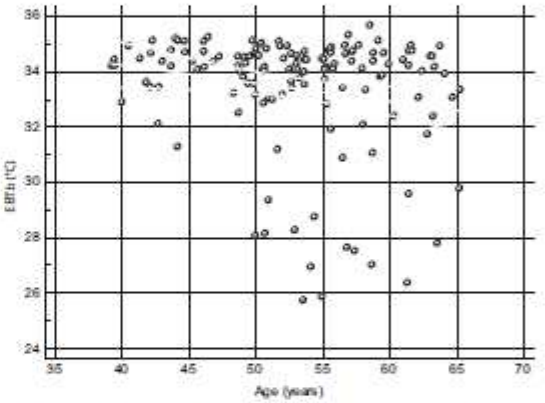

Figure 1. Association of EBTb with age (N = 140). Figure presents the association between the age (years) and the baseline exhaled breath temperature (EBTb), measured at the initial visit (Spearman R = −0.143, $p = 0.092$).

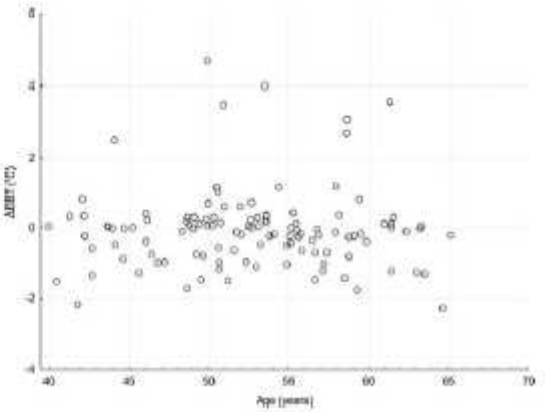

Figure 2. Association of ΔEBT with age (N = 140). Figure presents the association between the age (years) and the change in exhaled breath temperature after smoking cigarette (ΔEBT), measured at the initial visit (Spearman R = −0.069, $p = 0.448$).

Table 2. Characteristics of patients at the initial visit according to the pulmonologist assessment following GOLD guidelines (N = 140).

Variable*	Classification of patients				
	Asymptomatic (n = 37)	Symptomatic (n = 81)	COPD GOLD 1 (n = 22)	Statistics†	COPD LLN (n = 13)
Age (years)	52.2 ± 6.4	53.6 ± 6.4	52.1 ± 6.7	F = 0.876; p = 0.419	51.4 ± 5.7
Pack-years	36.6 ± 19.1	38.9 ± 17.9	39.6 ± 16.9	F = 0.255; p = 0.775	37.9 ± 15.9
PB FVC (SR)	0.76 ± 1.01	0.69 ± 1.08	0.86 ± 1.14	F = 0.219; p = 0.804	0.51 ± 1.24
PB FEV_1 (SR)	0.18 ± 0.98	0.25 ± 0.86	−0.62 ± 1.06	**F = 8.246; p < 0.001**	−1.09 ± 1.12
PB FEV_1/FVC (SR)	−0.26 ± 0.76	0.08 ± 0.71	−1.60 ± 0.44	**F = 53.879; p < 0.001**	−1.95 ± 0.30††
6MWT (%Pred)	63.74 ± 13.37	64.04 ± 14.26	66.15 ± 10.60	F = 0.186; p = 0.813	57.83 ± 12.43††
CAT score	5.2 ± 4.6	10.1 ± 6.7	13.1 ± 8.8	**H = 21.520; p < 0.001**	11.9 ± 5.8
SGRQ symptom score	12.3 ± 17.6	21.3 ± 16.3	30.5 ± 25.6	**H = 17.566; p < 0.001**	26.2 ± 19.0
SGRQ activity score	23.6 ± 15.2	26.0 ± 17.6	26.6 ± 19.9	**H = 15.628; p < 0.001**	23.8 ± 16.6
SGRQ impact score	3.7 ± 8.1	7.2 ± 9.1	11.5 ± 11.2	**H = 11.293; p = 0.004**	10.7 ± 9.4
SGRQ total score	8.2 ± 10.7	15.2 ± 10.4	19.2 ± 13.9	**H = 19.686; p < 0.001**	17.2 ± 10.0
EBTb (°C)	32.56 ± 3.66	33.98 ± 1.64	32.89 ± 3.47	F = 0.353; p = 0.703	33.96 ± 1.08
EBTc (°C)	32.66 ± 2.81	33.74 ± 1.71	32.82 ± 3.29	F = 1.248; p = 0.291	33.64 ± 0.88
ΔEBT (°C)	0.10 ± 2.00	−0.24 ± 0.63	−0.07 ± 1.48	F = 0.001; p = 0.999	−0.25 ± 1.33

Legend: *all values are presented as mean ± SD; †statistical significance for comparison between three groups according to GOLD was tested using analysis of variance (ANOVA, *F* value) or Kruskal-Wallis ANOVA (*H* value); ††significantly different to COPD GOLD 1 ($p < 0.05$); COPD: chronic obstructive pulmonary disease; GOLD: Global Initiative for Chronic Obstructive Lung Disease; PB – postbronchodilator value; FVC – forced vital capacity as standardized residuals (SR); FEV_1 – forced expiratory volume in 1 second as standardized residuals (SR); FEV_1/FVC as standardized residuals (SR); 6MWT – 6-minute walk test as % predicted (%Pred); CAT – COPD assessment test; SGRQ – St. George Respiratory Questionnaire; EBT – exhaled breath temperature; EBTb – baseline EBT; EBTc – EBT after smoking cigarette; ΔEBT – change in EBT after smoking cigarette.

Subgroups comparisons at initial visit

Subgroup (after the initial pulmonologist assessment) comparisons at initial visit are presented in Table 2. No significant differences were found for age, smoking exposure or functional exercise capacity between the subgroups ($p > 0.400$ for all). Also no significant difference was found for FVC (as SR, $p = 0.804$) but a significant difference was found for FEV_1 and FEV_1/FVC (expressed as SR, $p < 0.001$ for both) with worst results found as expected in GOLD 1 subgroup (as FEV_1/FVC < 0.7 was used to define this group). Comparable results were found for HRQoL data with the worst results found in GOLD 1 subgroup ($p < 0.01$ for all). No significant differences were found between these subgroups for EBT results (EBTb, EBTc and ΔEBT) ($p > 0.200$). In Table 2 we also presented the data for the subgroup of subjects diagnosed as COPD according to LLN criteria. Upon comparison with the COPD GOLD 1 subgroup, expected significantly lower values were found in this subgroup for FEV_1/FVC (expressed as SR, $p < 0.05$) and for the 6MWT (expressed as % predicted, $p < 0.05$). Other variables (age, smoking habit, FVC and FEV_1, HRQoL, EBT) were not significantly different between these two subgroups.

Study outcomes

Reassessment after 2 years of follow-up (average follow-up was 2 years and 54 days) for the predefined outcomes of DP showed that out of 118 patients without COPD, 7 (5.9%) patients had an ND COPD (rate per 100 person-years of 2.767, 95% CI 1.210–5.473) with additional 4 [11 (7.9%) altogether] that had progressed from COPD GOLD 1 stage to GOLD 2 stage (DP rate per 100 person-years of 3.667, 95% CI 1.928–6.373), and 60 (42.9%) had an increased rate of LoLF (>70 mL/year for the PB FEV_1). Using the outcome measures based on LLN (ND COPD and DP) or SR (LoLF), 5 patients had an ND COPD, 9 had DP and 14 had a loss of $FEV_1 > 1$ SR. Comparisons of three EBT markers (EBTb, EBTc and ΔEBT) between the subgroups according to the progression of the disease after 2-year follow-up (based on three predefined study outcome measures) and the ROC curve analyses are shown in Tables 3 and 4.

The results for the primary outcome showed that ΔEBT measured at initial visit had a marginal discriminative power for ND COPD according to the GOLD criteria ($p = 0.148$) with the increase in the signal when ATS/ERS criteria (LLN) was used ($p = 0.036$) giving an AUC of 0.859 (95% CI, 0.781–0.917;

Table 3. EBT (EBTb, EBTc and ΔEBT) according to the outcomes of disease progression after 2-year follow-up (N = 140).

Disease progression outcome			EBTb (°C)	EBTc (°C)	ΔEBT (°C)
ND COPD	GOLD	No (n = 111); Yes (n = 7); Statistics†	33.41 ± 2.17; 31.02 ± 4.14; Z = 1.419, p = 0.156	33.18 ± 2.21; 32.56 ± 2.80; Z = 0.601, p = 0.548	−0.20 ± 1.23; 1.54 ± 2.85; Z = 1.446, p = 0.148
	LLN	No (n = 113); Yes (n = 5); Statistics†	33.43 ± 2.23; 30.17 ± 3.03; **Z = 2.348, p = 0.019**	33.25 ± 2.10; 30.98 ± 3.87; Z = 1.518, p = 0.129	−0.28 ± 1.05; 2.72 ± 2.43; **Z = 2.099, p = 0.036**
DP	GOLD	No (n = 129); Yes (n = 11); Statistics†	33.51 ± 2.05; 31.29 ± 3.85; Z = 1.758, p = 0.079	33.27 ± 2.05; 32.71 ± 2.63; Z = 0.320, p = 0.749	−0.21 ± 1.21; 1.46 ± 2.51; **Z = 2.170, p = 0.030**
	LLN	No (n = 131); Yes (n = 9); Statistics†	33.51 ± 2.11; 31.01 ± 3.39; **Z = 2.249, p = 0.024**	33.35 ± 1.96; 31.45 ± 3.19; **Z = 2.175, p = 0.030**	−0.16 ± 1.30; 1.12 ± 2.42; Z = 1.827, p = 0.067
Increased LoLF	Loss of FEV_1 > 70 ml/year	No (n = 80); Yes (n = 60); Statistics†	33.49 ± 2.05; 33.23 ± 2.56; Z = 0.543, p = 0.587	33.20 ± 2.18; 33.29 ± 2.02; Z = 0.020, p = 0.984	−0.20 ± 1.34; 0.13 ± 1.57; Z = 0.104, p = 0.917
	Loss of FEV_1 > 1 SR	No (n = 126); Yes (n = 14); Statistics†	33.35 ± 2.32; 33.18 ± 2.18; Z = 0.802, p = 0.422	33.25 ± 2.18; 33.02 ± 2.49; Z = 0.159, p = 0.874	−0.07 ± 1.47; −0.10 ± 0.96; Z = 0.059, p = 0.953

Legend: ND COPD – newly diagnosed COPD after 2 years of follow-up; DP – disease progression – ND COPD + progression of a severity of COPD from GOLD 1 stage during the 2-year follow-up; LoLF – loss of lung function; GOLD: Global Initiative for Chronic Obstructive Lung Disease; LLN: lower level of normal; FEV_1: Forced expiratory volume in 1 second; SR: standardized residuals; EBT – exhaled breath temperature; EBTb – baseline EBT; EBTc – EBT after smoking cigarette; ΔEBT – change in EBT after smoking cigarette; *values are presented as mean ± SD; †statistical significance was tested using Mann-Whitney U-test.

Table 4. EBT (EBTb, EBTc and ΔEBT) ROC curve analysis data according to the outcomes of disease progression after 2-year follow-up (N = 140).

Disease progression outcome			EBTb	EBTc	ΔEBT
ND COPD	GOLD	AUC, 95% CI, Statistics	0.664, 0.571–0.748, $Z = 1.199$, $p = 0.231$	0.568, 0.466–0.667, $Z = 0.547$, $p = 0.584$	0.669, 0.568–0.759, $Z = 1.409$, $p = 0.159$
	LLN	AUC, 95% CI, Statistics	0.788, 0.703–0.858, **$Z = 2.330$, $p = 0.020$**	0.704, 0.605–0.791, $Z = 1.124$, $p = 0.261$	0.859, 0.781–0.917, **$Z = 2.533$, $p = 0.011$**
DP	GOLD	AUC, 95% CI, Statistics	0.663, 0.579–0.740, $Z = 1.595$, $p = 0.111$	0.530, 0.438–0.621, $Z = 0.274$, $p = 0.784$	0.711, 0.622–0.789, **$Z = 2.404$, $p = 0.016$**
	LLN	AUC, 95% CI, Statistics	0.716, 0.634–0.789, **$Z = 2.246$, $p = 0.025$**	0.731, 0.644–0.808, **$Z = 1.993$, $p = 0.046$**	0.614, 0.520–0.702, $Z = 0.783$, $p = 0.433$

Legend: ND COPD – newly diagnosed COPD after 2 years of follow-up; DP - disease progression – ND COPD + progression of a severity of COPD from GOLD 1 stage during the 2-year follow-up; GOLD: Global Initiative for Chronic Obstructive Lung Disease; LLN: lower level of normal; AUC: area under the curve; CI: confidence interval; EBT – exhaled breath temperature; EBTb – baseline EBT; EBTc – EBT after smoking cigarette; ΔEBT – change in EBT after smoking cigarette; statistical significance was tested using z statistics.

z statistic = 2.533, $p = 0.011$). A cut-off value of 1°C had a sensitivity of 66.7%, specificity of 98.1%, PPV of 75% and NPV of 97.3%. We found a significant discriminative power for DP according to the GOLD criteria ($p = 0.030$) with a comparative signal when ATS/ERS criteria (LLN) was used ($p = 0.067$) giving an AUC (GOLD criteria) of 0.711 (95% CI, 0.622–0.789; z statistic = 2.404, $p = 0.016$). A cut-off value of 0.17°C had a sensitivity of 60.0%, specificity of 75.0%, PPV of 17.7% and NPV of 95.5%. No significant difference was found for ΔEBT for the third outcome measure LoLF ($p > 0.9$ for both; Table 3).

Results for the secondary outcomes showed that EBTb was significantly discriminative for patients with two (out of three) outcome measures (ND COPD and DP) but only if ATS/ERS criteria were used (LLN) for the definition of airway limitation ($p = 0.019$ and $p = 0.024$, respectively; Table 3) giving moderately good results using ROC curve analysis (AUC = 0.788 and AUC = 0.716, respectively; Table 4) with EBTb at initial visit significantly lower in patients with the progression of disease. Results for the EBTc showed the same pattern but were significantly discriminative only for DP when ATS/ERS criteria were used (LLN) for the definition of airway limitation ($p = 0.030$; Table 3) giving a moderately good result using ROC curve analysis (AUC = 0.731; Table 4). Both EBTb and EBTc have not showed a significant difference for LoLF ($p > 0.87$ for all; Table 3).

Logistic regression analysis for ND COPD and DP using ATS/ERS criteria (LLN) taking into account sex, age, smoking exposure, lung function, 6MWT and EBT was carried out to assess markers of susceptibility to cigarette smoke for the progression of disease. For ND COPD, only ΔEBT was significantly associated with this outcome and had an OR equal to 1.74 (95% CI, 1.09–2.78; $p = 0.021$). For DP EBTb, PB FVC and PB FEV_1 (expressed as SR) were significantly associated with this outcome (EBTb OR = 0.71, 95% CI 0.54–0.92, $p = 0.010$; PB FVC OR = 13.34, 95% CI 1.79–99.38, $p = 0.012$; PB FEV_1 OR = 0.06, 95% CI 0.01–0.50, $p = 0.010$).

Discussion

The main result of this preliminary analysis is the potential of an average equilibrium exhaled breath temperature (EBTb and ΔEBT) measured at a single time point (at initial visit) to predict future (after 2-year follow-up) development of COPD or progression of disease with the increase in signal and more consistent results when using a more stringent ATS/ERS criteria for airway limitation (LLN). A much clearer signal when using LLN for diagnosing COPD is especially important in determining whether GOLD is a meaningful method for diagnosing COPD. These results were further corroborated by the results of logistic regression analysis (taking into account also sex, age, smoking, lung function, 6MWT) showing that ΔEBT was the only significant factor predicting ND COPD (using LLN) with 74% more odds for ND COPD with an increase in EBT per°C after smoking cigarette. Logistic regression analysis revealed that EBTb together with PB FVC and PB FEV_1 (both expressed as SR) were significant independent predictors for DP, but confidence intervals for lung function indexes were wide. On the other hand, EBT was not predictive for the LoLF either as absolute value or SR values (recommended for the lung function follow-up studies by ATS/ERS to exclude age-related bias especially).

As cigarette smoking represents the most significant single cause of death and disability in developed countries, as in the pathogenesis of COPD, the results of our study show a potential for the future research in this area – both for new markers of an early diagnosis and progression of COPD as well as for the possible need to revise the current GOLD criteria for diagnosing COPD. Need for further research is emphasized by COPD disease characteristics: COPD developed in less than one-third of smokers during their lifetime, preventive measures being difficult to implement with a low rate of success, progressive disease with an unsatisfactory treatment success, high public health impact with high morbidity, disability and early mortality rates. Thus, identification of individuals with a significant susceptibility to cigarette smoke and future development of COPD is still an important (major) research goal (2). Data analysis for EBTb and ΔEBT showed a moderate sensitivity and specificity for two of three outcomes ('newly diagnosed COPD' and 'newly diagnosed COPD + progression of severity of COPD'). This allows moderately differentiating patients at risk as either 'healthy' smokers or as patients with a significant potential to develop COPD in future or patients that will have a DP, with the potential for an early intervention.

EBT is a biological marker for which research was started during last decades especially as a noninvasive measure of airways inflammation in children and adults (30,31). Higher EBT found in asthmatics compared to healthy individuals was understood to be an indicator of airways inflammation, while at the same time axillary and ear temperature were comparable between groups supporting the fact that EBT was representing a local inflammatory process (25). Measurement methods used

in early research (instantaneous EBT measurement) produced EBT results that were closely associated with age, lung volume, type of breathing and environmental factors (room temperature and humidity), and so the results had to be adjusted for these factors (32). In our study we used the newer method developed by Popov et al. (X-halo® device), measuring EBT at the maximal plateau of stabilization (average equilibrium EBT) with spontaneous breathing pattern because measurements done using this method were not influenced by the pattern of breathing, lung volume and environmental factors, while the discriminating power of the method was preserved (15, 33, 34). The EBT results in our study show a wider range of results than other studies but patients were closely monitored during the measurements; we used a one-way valve, and when in doubt regarding the measured value, the measurement was repeated. To exclude further artifacts, patients ($n = 6$) with EBTb < 25°C were excluded. Also, the change in a breathing technique of the patient (breathing in through mouth instead of through nose or rebreathing through the device) can change the final result by less than 0.5°C which could not explain this wide range (unpublished results). As we had several devices, subjects did not use the same device one after another, excluding the possibility that the measurement from the previous subject could influence the results of the other. So the difference in the scatter of EBT values was most probably the consequence of the studied population which was different from other studies.

Previous research showed that the change in EBT during expiration (change from environmental temperature to the plateau) was lesser and slower in patients with COPD when compared to healthy controls, possibly as a consequence of mucus hypersecretion, remodeling and/or reduction in blood vasculature (35). This data, together with the fact that this change was not significantly increased after the inhalation of vasodilator drug (e.g. salbutamol), supports the concept of decreased vascularity of airways in COPD compared to patients with asthma (30, 36). Mean (±SD) EBT in our study was 33.33 ± 2.30°C, which was comparable to similar studies (37, 38). When we compared the subgroups of smokers according to symptomatic status and diagnosis of COPD, although we found the highest temperature in the subgroup of symptomatic smokers (33.98°C) and lowest in the subgroup of asymptomatic smokers (32.56°C), the difference did not reach statistical significance because of the significant overlap. As symptoms represent an important specific facet in COPD (different from lung function), as recognized in recent GOLD document, the association of symptoms with EBT found in our study might be important and deserves further research. The other reason for the association observed between EBT and symptoms could be that these subgroups represent different mixtures of phenotypes regarding airways inflammation and vascularity changes associated with the effect of cigarette smoke on the airways.

Spirometry with a bronchodilator test with the cut-off value of FEV_1/FVC of < 0.7 still represents the 'gold standard' for the COPD diagnosis according to GOLD initiative, although this single cut-off value was challenged by ATS/ERS recommendations for criteria in support of airway limitation using LLN, especially for patients older than 70 years of age (shown very clearly by the results of GLI (19)). The unique criterion (FEV_1/FVC < 0.7) for airway limitation yields a significant overestimation of COPD in older populations. Because of that, we used both criteria and showed that using more stringent criteria, although reducing the number of subjects with outcomes (lowering the statistical power), can enhance the 'signal'. This is an important finding that challenges the globally accepted GOLD criteria for the diagnosis of COPD. On the other hand, our study evaluated EBT as a marker of susceptibility to a cigarette smoke in a population at risk having already significant cigarette smoke exposure (active smokers with a smoking history of >20 pack-years), and as a possible predictor of future COPD and DP based on the single time-point measurement. This was evaluated using baseline EBT (EBTb) measurement, the EBT measurement after smoking the cigarette (EBTc), and the change in EBT after smoking cigarette (ΔEBT) to additionally evaluate the effect of cigarette smoke on EBT. Although the data is preliminary and needs further evaluation regarding the larger patient sample and longer follow-up, it showed a potential for the predictive power of EBT measured at single time point for the future development of COPD. High negative predictive value (>95%) gives the physician assurance that individuals with a risk for the development of COPD and a negative test do not have a specific susceptibility to cigarette smoke regarding the development and progression of COPD. This was however not associated with the rate of LoLF in our study. This could be explained by a significant variability of lung function measurements during this relatively short follow-up, thus significantly influencing measured trend.

Although the predictive power of EBTb and ΔEBT was somewhat comparable for COPD DP based on the results of our study, associations found using logistic regression analysis showed a somewhat different pattern for these two EBT markers; so further research in regard to the underlying mechanisms behind these two markers is needed. The results of our study that lower EBTb represents a higher risk for DP challenge the hypothesis that inflammation is the single driver of DP under the smoke exposure (at least in this population); therefore other possible mechanisms should also be taken into account (39, 40). The results of our study corroborated by the previously published data support the potential of EBT as a possible marker of susceptibility to cigarette smoke and future development and progression of COPD. The limitations of our study were: relatively small sample size and limited follow-up time, in particular related to the low COPD progression rate found in our cohort. Further research is needed in this and other comparative populations at risk to complete the validation of this method.

Conclusions

Results of this preliminary analysis of the 2-year follow-up of a cohort of patients at risk for COPD have shown the potential of EBT in identifying patients susceptible to cigarette smoke, which would develop into clinically manifest COPD or would have a progression of disease with a better signal using LLN as criteria for COPD diagnosis. This is the first time that we have a potential biomarker measured at a single time point that can potentially predict future development of COPD. As this preliminary data analysis had a limited number of patients who were followed for 2 years with a low rate of DP, these results need additional confirmation in a larger sample with

a longer follow-up time including other comparative populations as well. If these results are confirmed in future studies, this could allow new preventive actions to be developed in patients at risk for COPD, thereby possibly lowering the burden of the disease.

List of abbreviations

ANOVA	analysis of variance
ATS	American Thoracic Society
AUC	area under the curve
BMI	body mass index
CAT	COPD Assessment Test
CI	confidence interval
COPD	chronic obstructive pulmonary disease
CRP	C-reactive protein
CVI	cerebrovascular infarction
DP	disease progression
EBT	exhaled breath temperature
EBTb	baseline EBT
EBTc	EBT after smoking cigarette
ΔEBT	change in EBT after smoking cigarette
ERS	European Respiratory Society
FEV_1	forced expiratory volume in 1. second
FVC	forced vital capacity
GCP	Good Clinical Practice
GLI	Global Lung Initiative
GOLD	Global Initiative for Chronic Obstructive Lung Disease
GP	general practitioner
HR	health related
hs	highly sensitive
LLN	lower level of normal
LoLF	rate of loss of lung function
MI	myocardial infarction
ND	newly diagnosed
NPV	negative predictive value
PB	postbronchodilator
PEF	peak expiratory flow
PPV	positive predictive value
QoL	Quality of Life
ROC	receiver operator curve
SD	standard deviation
SGRQ	Saint George Respiratory Questionnaire
SR	standardized residuals
TIA	transient ischemic attack
WBC	white blood cell count
6MWT	6-minute walk test
%Pred	% of a predicted value.

Declaration of interests

The authors declare that they have no conflict of interests. This study was part of MARKO study. Prof. Davor Plavec and Dr. Žarko Vrbica as principal investigators and Children's Hospital Srebrnjak have initiated study MARKO (ClinicalTrials.gov Identifier NCT01550679) and received an unrestricted grant from GlaxoSmithKline. GlaxoSmithKline has not influenced the study design or study protocol, has no ownership rights over data gathered by the study and has no influence on this or further publications of data gathered throughout this study.

Funding

The study was in part funded by the unrestricted grant from GlaxoSmithKline (GSK ***eTrack number:*** CRT114338).

References

1. Repine JE, Bast A, Lankhorst I. Oxidative stress in chronic obstructive pulmonary disease. The Oxidative Stress Study Group. Am J Respir Crit Care Med 1997; 156(2 Pt 1):341–357.
2. Lokke A, Lange P, Scharling H, Fabricius P, Vestbo J. Developing COPD: a 25 year follow up study of the general population. Thorax 2006; 61(11):935–939.
3. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2020 to 2030. PLoS Med 2006; 3(11):e442.
4. Kim W, Ling SH, Coxson HO, English JC, Yee J, Levy RD, et al. The association between small airway obstruction and emphysema phenotypes in COPD. Chest 2007; 131(5):1372–1378.
5. Georgopoulos D, Anthonisen NR. Symptoms and signs of COPD. In: Chernlack NS, editor. Chronic Obstructive Pulmonary Disease. Toronto: WB Saunders, 1991; 357–363.
6. Greulich T, Koczulla R, Vogelmeier C, Bals R. Chronic obstructive pulmonary disease (COPD) as a systemic disease. Dtsch Med Wochenschr 2009; 134(23):1231–1235.
7. Antoniu SA. Descriptors of dyspnea in obstructive lung diseases. Multidiscip Resp Med 2010; 5(3):216–219.
8. Pena VS, Miravitlles M, Gabriel R, Jiménez-Ruiz CA, Villasante C, Masa JF, et al. Geographic variations in prevalence and underdiagnosis of COPD: results of the IBERPOC multicentre epidemiological study. Chest 2000; 118(4):981–989.
9. Talamo C, de Oca MM, Halbert R, Perez-Padilla R, Jardim JR, Muiño A, et al. PLATINO team. Diagnostic labeling of COPD in five Latin Americas cities. Chest 2007; 131(1):60–67.
10. Murray CJ, Lopez AD. Alternative projections of mortality and disability by cause 1990–2020: Global Burden of Disease Study. Lancet 1997; 349(9064):1498–1504.
11. Murray CJ, Lopez AD. The global burden of disease: a comprehensive assessment of mortality and disability from diseases, injuries and risk factors in 1990 and projected to 2020. The global burden of disease: a comprehensive assessment of mortality and disability from diseases, injuries and risk factors in 1990 and projected to 2020. Global Burden of Disease and Injury Series, vol. 1. Cambridge: Harvard University Press, 1996.
12. Paredi P, Ward S, Cramer D, Barnes PJ, Kharitonov SA. Normal bronchial blood flow in COPD is unaffected by inhaled corticosteroids and correlates with exhaled nitric oxide. Chest 2007; 131(4):1075–1081.
13. Eisner MD, Anthonisen N, Coultas D, Kuenzli N, Perez-Padilla R, Postma D, et al. Committee on nonsmoking COPD, environmental and occupational health assembly. An official American Thoracic Society public policy statement: Novel risk factors and the global burden of chronic obstructive pulmonary disease. Am J Resp Crit Care Med 2010; 182(5):693–718.
14. Popov TA, Kralimarkova TZ, Dimitrov DV. Measurement of exhaled breath temperature in science and clinical practice. Breathe 2012; 8(3):187–192.
15. Paredi P, Kharitonov SA, Barnes PJ. Correlation of exhaled breath temperature with bronchial blood flow in asthma. Resp Res 2005; 6(1):15.
16. Carpagnano GE, Lacedonia D, Spanevello A, Martinelli D, Saliani V, Ruggieri C, et al. Exhaled breath temperature in NSCLC: could be a new non-invasive marker? Med Oncol 2014; 31(5):952.
17. Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2015. Available from: http://www.goldcopd.org/ (accessed May, 2015).
18. Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC. Lung volumes and forced ventilatory flows. Report Working

Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. Eur Respir J 1993; 6(16):5–40.
19. Quanjer PH, Brazzale DJ, Boros PW, Pretto JJ. Implications of adopting the global lungs initiative 2012 all-age reference equations for spirometry. Eur Resp J 2013; 42:1046–1054.
20. Miller MR. Does the use of per cent of predicted have any evidence base? Eur Resp J 2015; 45:322–3.
21. Jones PW, Quirk FH, Baveystock CM, Littlejohns P. A self-complete measure for chronic airflow limitation - the St George's Respiratory Questionnaire. Am Rev Resp Dis 1992; 145(6):1321–1327.
22. Jones PW, Harding G, Berry P, Wiklund I, Chen WH, Kline Leidy N. Development and first validation of the COPD assessment test. Eur Resp J 2009; 34(3):648–654.
23. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. ATS/ERS Task Force. Standardisation of spirometry. Eur Resp J 2005; 26(2):319–338.
24. Quanjer PH, Stanojevic S, Cole TJ et al. and the ERS Global Lung Function Initiative. Multi-ethnic reference values for spirometry for the 3–95 years age range: the global lung function 2012 equations. Eur Resp J 2012; 40:1324–43.
25. Popov TA, Dunev S, Kralimarkova TZ, Kraeva S, DuBuske LM. Evaluation of a simple, potentially individual device for exhaled breath temperature measurement. Resp Med 2007; 101(10):2044–2050.
26. Xepapadaki P, Xatzioannou A, Chatzicharalambous M, Makrinioti H, Papadopoulos NG. Exhaled breath temperature increases during mild exacerbations in children with virus induced asthma. Int Arch Allergy Immunol 2010; 153(1):70–74.
27. Popov TA. Human exhaled breath analysis. Ann Allergy Asthma Immunol 2011; 106(6):451–456.
28. ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories. ATS statement: guidelines for the six-minute walk test. Am J Resp Crit Care Med 2002;166(1):111–117.
29. Troosters T, Gosselink R, Decramer M. Six minute walk distance in healthy elderly subjects. Eur Resp J 1999; 14(2):270–274.
30. Paredi P, Kharitonov SA, Barnes PJ. Faster rise of exhaled breath temperature in asthma: a novel marker of airway inflammation? Am J Resp Crit CareMed 2002; 165(2):181–184.
31. Piacentini GL, Peroni D, Crestani E, Zardini F, Bodini A, Costella S, et al. Exhaled air temperature in asthma: methods and relationship with markers of disease. Clin Exp Allergy 2007; 37(3):415–419.
32. Popov TA, Kralimarkova TZ, Lazarova CT, Tzachev CT, Dimitrov VD, Gill J. Daily monitoring of asthmatics by means of individual devices for exhaled breath temperature measurement. IEEE Sensors J 2010; 10(1):44–48.
33. Cole P. Recordings of respiratory temperature. J Laryngol Otol 1954; 68(5):295–307.
34. Paredi P, Kharitonov SA, Barnes PJ. Exhaled breath temperature in airways disease. Eur Resp J 2003; 22(2):394.
35. Paredi P, Caramori G, Cramer D, Ward D, Ciaccia A, Papi A, et al. Slower rise of exhaled breath temperature in chronic obstructive pulmonary disease. Eur Resp J 2003; 21(3):439–443.
36. Dela Cruz CS, Tanoue LT, Matthay RA. Lung cancer: epidemiology, etiology, and prevention. Clin Chest Med 2011; 32(4):605–644.
37. Bijnens E, Pieters N, Dewitte H, Cox B, Janssen BG, Saenen N, et al. Host and environmental predictors of exhaled breath temperature in the elderly. BMC Public Health 2013; 13:1226.
38. Melo RE, Popov TA, Solé D. Exhaled breath temperature, a new biomarker in asthma control: a pilot study. J Bras Pneumol 2010; 36(6):693–699.
39. Hamill LM, Ferris KCA, Kapand KM, McConagh LA, Shields MD. Is exhaled breath temperature the new asthma inflammometer. Arch Dis Child 2012; 97(1):A29–A30.
40. Agusti A, Edwards LD, Rennard SI, MacNee W, Tal-Singer R, Miller BE, et al. Persistent systemic inflammation is associated with poor clinical outcomes in COPD: a novel phenotype. PLoS ONE 2012; 7(5):e37483.

3.5. Breathlessness and "exacerbation" questions predictive for incident COPD (MARKO study): data after two years of follow-up.

Breathlessness and "exacerbation" questions predictive for incident COPD (MARKO study): data after two years of follow-up

Žarko Vrbica[1,2,*], Justinija Steiner[3,*], Marina Labor[4], Ivan Gudelj[5] and Davor Plavec[6,7]

[1] Medical Nursing, University of Dubrovnik, Dubrovnik, Croatia, Dubrovnik, Croatia
[2] Pulmonology and Immunology, Dubrovnik General Hospital, Dubrovnik, Croatia, Croatia
[3] Osijek-Baranja Country Medical Center, Osijek, Croatia, Osijek, Croatia
[4] Cancer and Lung Health Care Unit, University Hospital at Linköping, Linköping, Sweden
[5] Medical Faculty, University of Split, Split, Croatia
[6] Research Department, Prima Nova, Zagreb, Croatia
[7] Medical Faculty, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia
[*] These authors contributed equally to this work.

ABSTRACT

Aims: To determine the predictability of the MARKO questionnaire and/or its domains, individually or in combination with other markers and characteristics (age, gender, smoking history, lung function, 6-min walk test (6 MWT), exhaled breath temperature (EBT), and hsCRP for the incident chronic obstructive pulmonary disease (COPD) in subjects at risk over 2 years follow-up period).

Participants and Methods: Patients, smokers/ex-smokers with >20 pack-years, aged 40–65 years of both sexes were recruited and followed for 2 years. After recruitment and signing the informed consent at the GP, a detailed diagnostic workout was done by the pulmonologist; they completed three self-assessment questionnaires—MARKO, SGRQ and CAT, detailed history and physical, laboratory (CBC, hsCRP), lung function tests with bronchodilator and EBT. At the 2 year follow-up visit they performed: the same three self-assessment questionnaires, history and physical, lung function tests and EBT.

Results: A sample of 320 subjects (41.9% male), mean (SD) age 51.9 (7.4) years with 36.4 (17.4) pack-years of smoking was reassessed after 2.1 years. Exploratory factor analysis of MARKO questionnaire isolated three distinct domains (breathlessness and fatigue, "exacerbations", cough and expectorations). We have determined a rate for incident COPD that was 4.911/100 person-years (95% CI [3.436–6.816]). We found out that questions about breathlessness and "exacerbations", and male sex were predictive of incident COPD after two years follow-up (AUC 0.79, 95% CI [0.74–0.84], $p < 0.001$). When only active smokers were analyzed a change in EBT after a cigarette (ΔEBT) was added to a previous model (AUC 0.83, 95% CI [0.78–0.88], $p < 0.001$).

Conclusion: Our preliminary data shows that the MARKO questionnaire combined with EBT (change after a cigarette smoke) could potentially serve as early markers of future COPD in smokers.

Submitted 21 July 2023
Accepted 20 November 2023
Published 18 December 2023

Corresponding author
Davor Plavec,
davorplavec@gmail.com

Academic editor
Oyelola Adegboye

Additional Information and Declarations can be found on page 14

DOI 10.7717/peerj.16650

How to cite this article Vrbica Ž, Steiner J, Labor M, Gudelj I, Plavec D. 2023. Breathlessness and "exacerbation" questions predictive for incident COPD (MARKO study): data after two years of follow-up. PeerJ 11:e16650 DOI 10.7717/peerj.16650

Subjects Public Health, Respiratory Medicine
Keywords Pre-COPD, Early COPD, Health related quality of life (HRQOL), Questionnaire, Chronic obstructive pulmonary disease, Smoking habit, Predictive markers, Validity

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is one of the top three causes of death worldwide and represents an important public health challenge that is both preventable and treatable (*Mathers & Loncar, 2006*; *GOLD, 2023*). COPD is characterized by persistent airflow limitation that is typically progressive and associated with an enhanced chronic inflammatory response in the airways and lung tissue to harmful particles or gases (*Vestbo et al., 2013*).

Early detection and treatment of COPD can help manage symptoms and slow the progression of the disease. Because of that, GOLD advocates active case finding in patients with symptoms and/or risk factors, but not screening spirometry. Wide reference values for spirometry prevent earlier diagnosis and spirometry from being used for screening, and simple portable devices are not widely accepted for COPD case finding (*Labor et al., 2016b*). Finding patients with early COPD is already difficult, but even those diagnosed in an early stage of disease (based on spirometry criteria) have already an advanced disease in the biological point of view. Clinically, early COPD is in patients represented with a developed airflow limitation due to the widespread damage to the bronchi and lung parenchyma. To be able to prevent these pathohistological damage, we should detect the disease in the biologically early stage before the irreversible changes occurred (*Martinez et al., 2022*).

Cigarette smoking is a key environmental risk factor for COPD, but fewer than 50% of heavy smokers develop COPD (*Lundbäck et al., 2003*). Since not all smokers and not even all the patients with pre-COPD (individuals with structural lung lesions and/or physiological abnormalities but without airflow obstruction) and Preserved Ratio Impaired Spirometry (PRISm) will eventually develop overt COPD, it is important to find the predictive markers that can detect the patients with early pathophysiological changes and find the way to protect them from further progression to clinically manifest COPD.

As the measurement of the exhaled breath temperature (EBT) using a portable device was found easy, non-invasive and reliable for monitoring airway inflammation it was used in smokers and COPD for research (*Popov et al., 2017*). It was shown by a group of authors that EBT increases in acute exacerbation of COPD and may be related to airway inflammation (*Lázár et al., 2014*). Smoking has been found to cause an acute increase in airways inflammation but not in all smokers. Change in EBT after smoking a cigarette in patients without a diagnosis of COPD and in GOLD 1 stage at initial assessment was significantly predictive for disease progression after 2 years (*Labor et al., 2016a*). Thus it was shown that EBT could have a potential to predict the future development of COPD at least in a proportion of subjects (current smokers). This data still needs to be confirmed by future studies.

So one of the aims of the MARKO project (Early detection of COPD patients in GOLD 0 (smokers) population–MARKO project) (*Vrbica et al., 2017*) and this analysis was to

assess the predictability of MARKO questionnaire for incident COPD during the 2-years follow-up.

MATERIALS AND METHODS

The aim, design and setting of the study: This analysis is the part of the broader research project "Early Detection of COPD Patients in GOLD 0 (Smokers) Population–MARKO Project", a two-phase prospective observational cohort study in subjects at risk for COPD to identify individuals that will further on develop COPD. The MARKO project is a prospective, observational, non-interventional cohort study of subjects at risk for the development of COPD. The study was in detail described previously (*Vrbica et al., 2017*) and registered at https://clinicaltrials.gov/ct2/show/NCT01550679. The study was approved by the local Ethics Committee (CHS, 02/2009) and conducted according to the recent version of the Declaration of Helsinki, Good Clinical Practice (GCP), and other relevant international and national laws. All participants signed the informed consent form (ICF) before starting any procedure related to the study (*Vrbica et al., 2017*).

In brief the project was organized in two phases (Fig. 1). Phase I recruited 450 subjects with the risk of COPD by 25 GPs in and around four major cities, during any (unrelated to respiratory problems) visit, if they satisfied inclusion/exclusion criteria and signed ICF. Inclusion criteria were: smokers/ex-smokers; both sexes; aged 40–65 years at inclusion; smoking history ≥20 pack-years (calculated as a number of cigarettes smoked per day multiplied by the number of years of smoking divided by 20); and no previous diagnosis of COPD. Exclusion criteria were: any clinically relevant chronic disease significantly affecting HRQoL; immunosuppressive therapy; acute respiratory disease 4 weeks before inclusion; hospitalization during the past 3 months; myocardial infarction (MI), cerebrovascular infarction (CVI) or transient ischemic attack (TIA) during the past 6 months; diagnosis of asthma; and an inability to perform the diagnostic protocol. Subjects filled out the MARKO questionnaire and measured lung function at GP's office and were referred to pulmonologist. There a diagnostic workup consisting of the MARKO questionnaire, HRQoL questionnaires (SGRQ and CAT), history and physical, exhaled breath temperature (EBT) before (EBTb) and after a smoked cigarette (EBTc), lung function testing with bronchodilator (salbutamol), lung diffusion capacity (DLCO), blood sampling (hematology; highly sensitive C-reactive protein (hs-CRP)), 6-min walk test (6MWT), and the assessment for diagnosis and severity of COPD were done.

Phase II included subjects from phase I assessed as 'healthy' smokers, symptomatic smokers (GOLD 0) or as COPD GOLD 1 that were followed and reassessed after 2 years (±2 months) after baseline by the same pulmonologist. Incident COPD diagnosis was made by the same trained pulmonologist in a tertiary level hospital based on clinical presentation and lung function according to GOLD. Incidence of newly diagnosed COPD after 2-years of follow up was used to identify diagnostic parameters that are most sensitive for early impairment in COPD, to determine the predictability of developed screening MARKO questionnaire alone or with other markers of early impairment in COPD. The flow-chart of the MARKO study is presented in Fig. 1.

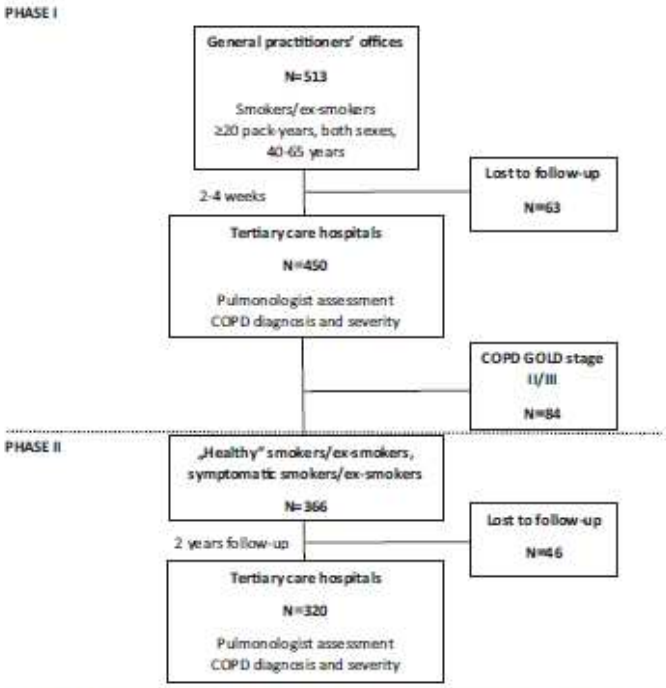

Figure 1 **Flow-chart of the MARKO study.** Full-size DOI: 10.7717/peerj.16650/fig-1

This research attempts to answer two following questions: (1) can we identify cheap and simple tools that will allow a precise and accurate identification of future incident COPD from a population at risk; (2) is there a combination (pattern) of tools, functional parameters, genetic and biochemical markers that can reliably predict incident COPD in a population at risk, thus allowing early intervention.

Primary end point of this analysis was to assess the predictability of newly constructed self-administered health related quality of life (HRQoL) questionnaire (MARKO questionnaire) and its domains to be used alone or in combination with other markers (exhaled breath temperature (EBT), lung function, inflammatory markers) in identification of subjects who will develop COPD during the 2-year follow-up.

Secondary endpoint: to determine the rate of progression of COPD in patients with GOLD 0 during the follow-up.

Methods

Screening questionnaire (MARKO questionnaire). The MARKO questionnaire is a newly constructed HRQoL questionnaire developed by a group of experts. The questionnaire and the psychometric characteristics were already published elsewhere (*Vrbica et al., 2016*). The questionnaire comprises 18 questions covering the manifestation and frequency of the symptoms already present at early stages of COPD (Supplemental Material). The total scores ranged 0 to 57 with higher scores indicating poorer HRQoL.

St. George Respiratory Questionnaire (SGRQ). The SGRQ is a standardized self-administered airways disease-specific questionnaire divided into three subscales: symptoms (eight items), activity (16 items), and impacts (26 items). SGRQ scores were calculated using the Excel® SGRQ calculator with scores ranging from 0 (no impairment) to 100 (maximum impairment) (*Jones et al., 1992*).

COPD Assessment Test (CAT). The CAT is a validated, short (eight-item) patient completed questionnaire developed for use in routine clinical practice to measure the health status of patients with COPD, with scores ranging from 0 (no impairment) to 40 (maximum impairment) (*Jones et al., 2009*).

Lung function. Spirometry was performed using computerized pneumotachographs (Jaeger®, CareFusion, San Diego, CA, USA) using the same procedure at all clinical sites (lung function labs at tertiary hospitals) in agreement with the ATS/ERS standardization (*Quanjer et al., 2012*). Bronchodilator test was done with repeated spirometry 20 min after inhalation of 400 mcg of salbutamol using the inhalation chamber. Spirometric parameters (FVC, FEV1, FEV1/FVC ratio, peak expiratory flow (PEF), forced expiratory flow between 25 and 75% FVC (FEF25-75)) were recorded as absolute values and as percentage of predicted according to GLI (*Quanjer et al., 2012*). Single-breath diffusing capacity of the lung for carbon monoxide (DLCO) was measured using a rapid carbon monoxide and helium analyzer (Ganzhorn, Germany), which was calibrated prior to each measurement. Values for DLCO and DLCO corrected for alveolar volume (VA) (DLCO/VA) were obtained and are reported as percent predicted values (*Roca et al., 1990*).

Exhaled breath temperature (EBT). EBT was measured using X-Halo® device (Delmedica Investments, Singapore) according to a previously validated method (*Popov et al., 2007*). EBT was measured twice on the same occasion (during the initial visit); (1) baseline measurement before any other procedure (lung function, bronchodilator test, 6 MWT) at least 1h after the last smoked cigarette (EBTb) and (2) done only in active smokers 15 min after a smoked cigarette (EBTc) and recorded with precision of 1/100 of a °C. No other procedure apart from cigarette smoking was carried out between the two EBT measurements.

Blood sampling. Blood samples for serum were drawn at minimal volume of 3 ml in serum separation vacuum tubes (containing Z Serum Clot Activator gel) and sent to the laboratory. Samples were kept at room temperature for at least 30 min, but had to be processed within 2 h of blood collection. Upon centrifugation at 3,000 rpm for 10 min, a minimum of 300 µl of serum was separated for further detection of hs-CRP level. Blood samples for hematology were drawn at minimal volume of 3 ml in K2EDTA vacuum blood collection tubes and sent to the laboratory for complete blood cell hematological analysis. Laboratory analyses were done in local laboratories and included complete blood count, white blood cells differential count, hematocrit, hemoglobin and hs-CRP.

Functional exercise capacity was assessed using 6 MWT according to the ATS guidelines and expressed as walked distance in meters and as % of predicted according to *Troosters, Gosselink & Decramer (1999)*.

Data analyses

Data analyses were done using STATISTICA version 12 (StatSoft, Inc., Tulsa, OK, USA), and MedCalc Statistical Software version 22 (MedCalc Software bvba, Ostend, Belgium; https://www.medcalc.org; 2023). Sample size calculation was done based on the following assumptions: we expected to find 25% patients in different stages of COPD according to the airflow limitation. We therefore expected that the sample would consist of 75% of 'healthy' and symptomatic smokers, 12.5% patients with COPD GOLD 1, 6.25% in GOLD 2 and 6.25% in GOLD 3 or 4 stages at initial visit with the expected difference in the MARKO questionnaire scores of two points and SD of 2.5 points between 'healthy' smokers *vs* symptomatic smokers *vs*. COPD GOLD 1/2 having a statistical power of >80% with alpha = 0.05 for the sample size of at least 500 subjects. The expected yearly incidence rate was planned at 10/per 100 patient-years.

Categorical data were presented as absolute numbers and percentages. Quantitative data were expressed as mean and standard deviation (SD) or median and interquartile range (IQR) with regards to the type of distribution. Normality of distribution was assessed using Kolmogorov-Smirnov test. Categorical data were compared between subgroups using chi-square test or Fisher exact test and continuous variables using Mann-Whitney U-test and Kruskal-Wallis ANOVA (non-normal distribution expected). Secondary validation of the MARKO questionnaire was done to determine the construct validity and predictability (alone or in combination with other markers) for future development and/or progression of COPD. Construct validity of the MARKO questionnaire was assessed using factorial analysis to confirm the number of factors with calculations of inter- and intracorrelations between the factors and items. Utility of different markers for disease progression was assessed using logistic regression models and expressed as odds ratio (OR) with 95% confidence intervals (CIs). Predictive power for the models was presented using receiver operator curve (ROC) analysis with AUC (with 95% CIs). $p < 0.05$ was used as statistically significant for all analyses with the correction for multiple comparisons.

RESULTS

Three hundred and twenty patients (186 women) aged 40–65 years at entry were included into this cohort study. Women had a mean (SD) age of 51.4 (7.1) years, and men 52.5 (7.8) years (t = 1.292, $p = 0.198$) with a mean smoking history of 31.7 (14.0) years and 42.9 (19.5) pack-years, men having a significantly greater cumulative exposure (t = 5.677, $p < 0.001$). BMI was 26.12 (4.51) kgm-2 in women and 27.42 (3.90) kgm-2 in men (t = 2.753, $p = 0.006$). Post BD FEV1/FVC was significantly greater in women than in men (mean ± SD; 0.79 ± 0.06 *vs* 0.77 ± 0.07, t = 3.087; $p = 0.002$), with comparative post BD FVC (98.0 ± 12.6% *vs* 97.3 ± 13.3%, t = 0.475; $p = 0.635$) and FEV1 (97.2 ± 14.4% *vs* 95.3 ± 15.1%, t = 1.132; $p = 0.259$). 6MWT (expressed as the % of predicted) was significantly lower in men (mean ± SD; 64.4 ± 10.8% *vs* 60.4 ± 13.3%, t = 2.867; $p = 0.005$).

In Table 1 results of the exploratory factor analysis are presented as factor loadings. Three distinct domains were found with first domain representing questions about different levels of breathlessness and fatigue, second domain representing questions about cough and phlegm and third domain representing questions about "exacerbations" (severe

Table 1 Factor analysis for MARKO questionnaire done at baseline ($N = 320$).

Variable	Factor loadings		
	Factor (1)	Factor (2)	Factor (3)
MQq 1	0.121	**0.865**	0.137
MQq 2	0.114	**0.850**	0.083
MQq 3	**0.573**	0.444	0.200
MQq 4	0.069	0.184	**0.860**
MQq 5	0.128	0.037	**0.878**
MQq 6	**0.688**	0.343	0.172
MQq 7	**0.636**	0.025	0.360
MQq 8	**0.712**	0.065	0.211
MQq 9	**0.771**	0.033	0.155
MQq 10	**0.726**	0.302	0.027
MQq 11	**0.687**	0.412	0.001
MQq 12	**0.663**	0.373	−0.075
MQq 13	**0.798**	0.128	0.119
MQq 14	**0.766**	0.029	0.065
MQq 15	**0.807**	0.129	0.024
MQq 16	**0.802**	0.048	0.040
MQq 17	**0.602**	0.366	0.021
MQq 18	**0.636**	0.390	0.016
Expl.Var	7.081	2.548	1.831
Prp.Totl	0.393	0.142	0.102

Note:
MQq, MARKO questionnaire question number; bold text represents significant factor loadings.

cold with cough or bronchitis and use of antibiotics for a severe cold with cough or bronchitis) during the past year.

Follow-up visit

Changes between baseline and follow-up visit are presented in Table 2. Not many changes were seen when the sample was assessed as a whole group. It can be seen that as expected smoking history was significantly greater ($p < 0.001$) after 2 years. Dyspnea (mMRC) was however significantly lower ($p < 0.001$) despite the fact that lung function was significantly worse ($p < 0.001$ for FVC, FEV1, and Tiff), except for post BD PEF ($p = 0.209$). The results of the MQ were significantly better ($p = 0.001$), but CAT score was comparable ($p = 0.238$). SGRQ activity score was worse ($p = 0.043$) and impact, symptoms and total scores were comparable ($p > 0.05$ for all).

Incident COPD

Incident COPD at the follow-up visit was determined in 33 subjects, with a rate of 4.911/100 patient-years (95% CI [3.436–6.816]). In Table 3 comparison of baseline characteristics between incident COPD group and other subjects is shown. There were significantly more males (75.8% *vs* 37.3%, $p < 0.001$) with a significantly higher smoking

Table 2 Comparison of baseline and follow-up visit data ($N = 320$).

Variables	Baseline		Follow-up		Paired differences			
	Mean	SD	Mean	SD	Mean	SD	95% CI	p^a
Body weight (kg)	79.04	16.11	79.05	16.73	0.01	4.93	[−0.54 to 0.55]	0.251
Smoking history (p/y)	36.32	17.45	38.12	18.05	1.81	2.11	[2.04–1.57]	<0.001
Comorbidities (No)	0.68	0.79	0.63	0.80	−0.05	0.80	[−0.14 to 0.04]	0.284
Chronic therapy (No)	0.84	1.14	0.77	1.25	−0.08	1.17	[−0.21 to 0.05]	0.144
mMRC	0.67	0.78	0.47	0.66	−0.20	0.79	[−0.29 to −0.11]	<0.001
Post BD FVC (L)	4.10	1.03	3.84	1.00	−0.27	0.44	[−0.31 to −0.22]	<0.001
Post BD FVC (% predicted)	97.71	12.94	92.85	13.88	−4.87	10.42	[−6.04 to −3.70]	<0.001
Post BD FVC (z-score)	−0.17	0.94	−0.52	1.00	−0.34	0.76	[−0.43 to −0.26]	<0.001
Post BD FEV1 (L)	3.19	0.80	3.06	0.78	−0.14	0.33	[−0.18 to −0.10]	<0.001
Post BD FEV1 (% predicted)	96.44	14.73	94.18	15.57	−2.25	9.72	[−3.34 to −1.16]	<0.001
Post BD FEV1 (z-score)	−0.25	1.08	−0.40	1.12	−0.15	0.73	[−0.23 to −0.07]	<0.001
Post BD TIFF (%)	78.22	6.76	80.14	7.60	1.92	5.55	[1.30–2.53]	<0.001
Post BD TIFF (% predicted)	98.29	8.27	101.10	9.37	2.81	7.01	[2.03–3.58]	<0.001
Post BD MEF25 (L/s)	1.17	0.58	1.66	5.86	0.49	5.87	[−0.17 to 1.16]	0.010
Post BD MEF50 (L/s)	3.87	1.48	3.75	1.49	−0.12	0.86	[−0.21 to −0.02]	0.002
Post BD PEF (L/s)	7.70	2.09	7.59	2.17	−0.11	1.40	[−0.27 to 0.05]	0.209
MARKO questionnaire (score)	13.10	9.08	11.97	8.42	−1.13	7.31	[−1.95 to −0.32]	0.001
CAT score	9.73	6.98	9.15	6.81	−0.58	7.05	[−1.37 to 0.21]	0.238
SGRQ activity score	23.72	19.82	22.59	18.28	−1.13	22.76	[−3.74 to 1.49]	0.583
SGRQ impact score	8.06	10.75	9.17	9.71	1.11	12.93	[−0.38 to 2.59]	0.043
SGRQ systems score	20.39	19.19	20.42	18.31	0.03	23.84	[−2.70 to 2.76]	0.756
SGRQ total score	14.85	12.68	15.20	11.51	0.35	14.72	[−1.35 to 2.05]	0.342

Notes:
[a] Wilcoxon test (paired samples).
p/y , pack-years of smoking; mMRC, modified Medical Research Council dyspnea scale; BD, bronchodilator; FVC, forced vital capacity; FEV1, forced expiratory volume in 1 s; TIFF, Tiffeneau index (FEV1/FVC); MEF25, mid-expiratory flow at 75% of FVC; MEF50, mid-expiratory flow at 50% of FVC; PEF, peak expiratory flow; CAT, COPD Assessment Test; SGRQ, St. George's respiratory questionnaire.

exposure (42.99 *vs* 35.52 pack-years, $p = 0.008$). As more males were present height and weight were also significantly larger ($p < 0.001$ and $p = 0.046$). Incident COPD subjects had significantly more obstruction at baseline with lower post BD values for FEV1, Tiffeneau index and MEF25 and MEF50 ($p < 0.001$ for all). Although DLCO (% predicted) was comparable, KCO (% predicted) was significantly lower ($p = 0.049$). 6MWT showed comparative results, together with dyspnea (mMRC score), EBT, hematology and hsCRP. There were also no difference for CAT score, SGRQ domains and total score and MARKO questionnaire total score. Significant differences were found for answers to question number 4 ($p = 0.014$) of MQ and marginal for questions 3 and 5 ($p = 0.064$ and $p = 0.050$).

Multivariate logistic regression was used to define the baseline variables that can predict incident COPD. The results were shown in Figs. 2 and 3. We found out that questions about breathlessness ("Have you experienced being breathless during preceding 3 months?") and "exacerbations" ("Have you had a severe cold with cough or bronchitis during preceding 12 months?"), and male sex were predictive of incident COPD after

Table 3 Comparison of baseline measures between the groups of incident COPD and rest (at follow-up visit) (N = 320).

Variable	No COPD (n = 287)		Incident COPD (n = 33)				
	Mean	SD	Mean	SD	Difference	95% CI	p^*
Age (yrs)	51.76	7.38	52.38	7.80	0.62	[−2.06 to 3.31]	0.561
Sex (male)	106	37.3%	25	75.8%			<0.001
Smoking history (p/y)	35.52	17.01	42.99	19.18	7.47	[1.23–13.71]	0.008
Time from baseline (yrs)	2.11	0.21	2.14	0.22	0.03	[−0.05 to 0.11]	0.483
Body height (cm)	171.08	9.25	177.76	9.46	6.68	[3.32–10.04]	<0.001
Body weight (kg)	78.45	15.92	84.24	17.37	5.79	[−0.03 to 11.61]	0.046
BMI (kgm^{-2})	26.69	4.32	26.52	4.45	−0.17	[−1.74 to 1.40]	0.841
Heart rate (min^{-1})	77.76	12.26	76.79	13.22	−0.97	[−5.80 to 3.85]	0.788
Systolic blood pressure (mmHg)	127.26	14.50	131.43	12.39	4.17	[−1.42 to 9.76]	0.123
Diastolic blood pressure (mmHg)	80.38	9.07	80.57	9.08	0.19	[−3.35 to 3.73]	0.893
Commorbidities (No)	0.69	0.78	0.64	0.86	−0.05	[−0.34 to 0.24]	0.599
Chronic treatments (No)	0.86	1.13	0.82	1.26	−0.04	[−0.45 to 0.38]	0.591
Post BD FVC (L)	4.04	1.00	4.66	1.15	0.62	[0.25–0.99]	0.003
Post BD FVC (% predicted)	97.74	12.83	97.47	14.01	−0.26	[−5.03 to 4.50]	0.760
Post BD FVC (z score)	−0.17	0.92	−0.19	1.05	−0.02	[−0.36 to 0.33]	0.749
Post BD FEV1 (L)	3.20	0.80	3.13	0.85	−0.07	[−0.37 to 0.22]	0.795
Post BD FEV1 (% predicted)	97.95	13.94	83.35	15.11	−14.60	[−19.77 to −9.43]	<0.001
Post BD FEV1 (z score)	−0.14	1.02	−1.21	1.12	−1.07	[−1.45 to −0.69]	<0.001
Post BD TIFF (%)	79.51	5.62	67.21	5.55	−12.30	[−14.33 to −10.27]	<0.001
Post BD TIFF (% predicted)	99.81	6.89	85.24	7.60	−14.58	[−17.10 to −12.05]	<0.001
Post BD TIFF (z score)	0.01	0.86	−1.67	0.83	−1.68	[−1.99 to −1.37]	<0.001
Post BD MEF25 (L/s)	1.21	0.58	0.79	0.35	−0.42	[−0.63 to −0.20]	<0.001
Post BD MEF50 (L/s)	4.02	1.43	2.53	1.22	−1.50	[−2.02 to −0.97]	<0.001
Post BD PEF (L/s)	7.66	2.08	8.01	2.18	0.35	[−0.43 to 1.13]	0.199
DLCO (% predicted)	78.16	18.26	75.53	33.23	−2.63	[−10.76 to 5.50]	0.083
KCO (% predicted)	79.30	19.39	71.31	23.64	−7.99	[−16.02 to 0.04]	0.049
6 MWT (m)	442.39	88.85	433.81	100.29	−8.57	[−44.38 to 27.24]	0.763
6 MWT (%)	63.28	11.77	58.25	13.59	−5.03	[−9.79 to −0.28]	0.056
EBTb (°C)	33.01	2.83	32.43	3.37	−0.58	[−1.70 to 0.53]	0.404
EBTd (°C)	−0.06	1.46	0.40	1.92	0.46	[−0.16 to 1.08]	0.521
RBC	4.69	0.42	4.71	0.41	0.03	[−0.14 to 0.20]	0.626
Hgb	142.27	13.12	145.96	12.98	3.69	[−1.73 to 9.12]	0.208
htc	0.43	0.05	0.43	0.03	0.01	[−0.01 to 0.03]	0.179
WBC	8.26	1.97	8.10	2.04	−0.16	[−0.98 to 0.66]	0.639
hsCRP	3.32	4.03	2.80	2.15	−0.51	[−2.20 to 1.17]	0.769
mMRC	0.67	0.77	0.73	0.84	0.06	[−0.22 to 0.34]	0.770
SGRQ activity score	23.36	20.00	25.71	18.62	2.35	[−5.06 to 9.76]	0.463
SGRQ impact score	7.66	10.66	9.56	10.43	1.91	[−2.06 to 5.88]	0.136
SGRQ symptom score	19.54	18.42	25.79	23.60	6.25	[−0.84 to 13.34]	0.290
SGRQ total score	14.42	12.63	17.15	12.80	2.73	[−1.99 to 7.45]	0.163

(Continued)

Table 3 (continued)

Variable	No COPD (n = 287)		Incident COPD (n = 33)				
	Mean	SD	Mean	SD	Difference	95% CI	p^*
CAT (score)	9.46	6.85	12.07	7.77	2.61	[−0.01 to 5.23]	0.052
MQq 1	1.40	1.35	1.91	1.55	0.50	[0.00–1.01]	0.099
MQq 2	1.22	1.32	1.59	1.52	0.37	[−0.12 to 0.87]	0.207
MQq 3	0.63	1.00	1.06	1.32	0.44	[0.06–0.82]	0.064
MQq 4	0.42	0.54	0.66	0.55	0.24	[0.04–0.43]	0.015
MQq 5	0.31	0.49	0.50	0.57	0.19	[0.00–0.37]	0.050
MQq 6	0.63	0.63	0.75	0.67	0.12	[−0.12 to 0.35]	0.331
MQq 7	0.27	0.58	0.28	0.58	0.02	[−0.20 to 0.23]	0.799
MQq 8	0.25	0.53	0.25	0.51	0.00	[−0.20 to 0.19]	0.951
MQq 9	0.24	0.51	0.25	0.44	0.01	[−0.17 to 0.20]	0.574
MQq 10	0.56	0.73	0.69	0.97	0.13	[−0.15 to 0.40]	0.751
MQq 11	1.19	0.91	1.28	0.89	0.10	[−0.24 to 0.43]	0.602
MQq 12	1.30	0.96	1.38	0.79	0.07	[−0.28 to 0.42]	0.604
MQq 13	0.41	0.73	0.56	0.80	0.15	[−0.12 to 0.42]	0.148
MQq 14	0.33	0.62	0.31	0.47	−0.02	[−0.25 to 0.20]	0.730
MQq 15	0.57	0.73	0.63	0.71	0.05	[−0.21 to 0.32]	0.546
MQq 16	0.41	0.70	0.41	0.61	0.00	[−0.26 to 0.25]	0.774
MQq 17	1.42	0.78	1.34	0.65	−0.08	[−0.36 to 0.20]	0.387
MQq 18	1.32	0.80	1.38	0.71	0.05	[−0.23 to 0.34]	0.900
MQ (total score)	12.89	8.89	15.22	10.32	2.33	[−1.00 to 5.65]	0.277

Notes:

* Mann-Whitney test.

p/y , pack-years; BMI, body mass index; HR, heart rate; BD, bronchodilator; FVC, forced vital capacity; FEV1, forced expiratory volume in 1 s; TIFF, Tiffeneau index (FEV1/FVC); MEF25, mid-expiratory flow at 75% of FVC; MEF50, mid-expiratory flow at 50% of FVC; PEF, peak expiratory flow; DLCO, diffusing capacity of the lungs for carbon monoxide; KCO, carbon monoxide transfer coefficient; 6MWT, 6-min walk test; EBTb, baseline exhale breath temperature; EBTd, difference in exhaled breath temperature after smoking a cigarette; RBC, red blood cell count; Hgb, hemoglobin; htc, hematocrit; WBC, white blood cell count; hsCRP, high-sensitivity C-reactive protein; mMRC, modified Medical Research Council dyspnea scale; CAT, COPD Assessment Test; SGRQ, St. George's respiratory questionnaire; MQ, MARKO questionnaire; MQq, MARKO questionnaire question number.

2 years follow-up (AUC 0.79, 95% CI [0.74–0.84], $p < 0.001$) (Fig. 2). A 55% increase in odds for incident COPD was found for an increase in score to question 3, and an 243% increase in odds for increase in score to question 4 with the 11 times increased odds in male subjects.

When only active smokers were analyzed a change in EBT after a cigarette (ΔEBT) was added to a previous model (AUC 0.83, 95% CI [0.78–0.88], $p < 0.001$) (Fig. 3). A 29% of increase in odds for each 0.01 °C of EBTd, 69% increase in odds for incident COPD was found for an increase in score to question 3, and an 333% increase in odds for increase in score to question 4 with the almost 11 times increased odds in male subjects.

DISCUSSION

Aside from smoking cessation for active smokers, there are no disease modifying therapies for COPD (*Sin, 2023*). By the time patients develop spirometric obstruction, they have lost nearly half of their small airways and a third of their gas-exchanging units (*Koo et al., 2018*)

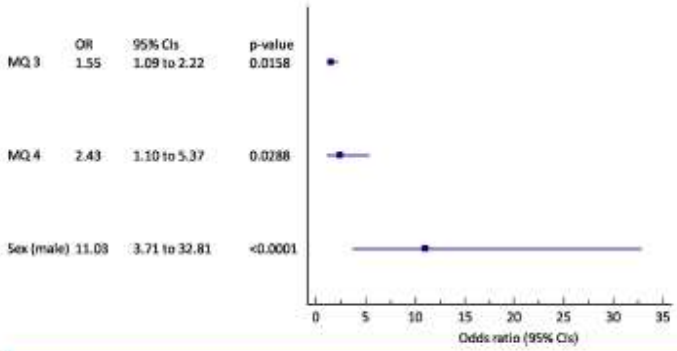

Figure 2 Forest plot of the results of the multivariate logistic regression for the prediction of incident COPD (N = 320). MQ, MARKO questionnaire question number; CIs, confidence intervals.
Full-size DOI: 10.7717/peerj.16650/fig-2

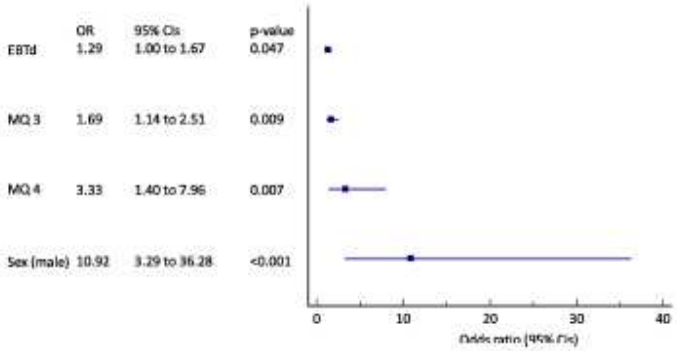

Figure 3 Forest plot of the results of the multivariate logistic regression for the prediction of incident COPD in active smokers (N = 245). EBTd, change in exhaled breath temperature after a smoked cigarette; MQ, MARKO questionnaire question number; CIs, confidence intervals.
Full-size DOI: 10.7717/peerj.16650/fig-3

and their disease is "fixed" and cannot be modified (*Stolz et al., 2022*). As lung regeneration is not possible at this point, it is crucial to identify "COPD" patients before they develop airflow limitation on spirometry and intervene at these early stages of disease. The importance of this stage is recently recognized and has different labels as "pre-COPD" and "early COPD" (*Celli et al., 2022*). No matter how we call it, for disease modification patients have to be identified at this stage of disease. More sensitive technologies (CT and hyperpolarized gas MRI, impulse oscillometry, body plethysmography and cardiopulmonary exercise tests) are required to make this diagnosis. That would implicate to test all the symptomatic smokers, but in the studies, up to 50% of smokers have some symptoms (*Woodruff et al., 2016*) but only 8% of them will develop COPD so better stratification of smokers at risk is needed.

Clinical course of COPD is often complicated by exacerbations and episodes of worsening of respiratory symptoms which contribute to disease progression. The

importance of COPD exacerbations cannot be overstated. The latest GOLD guidelines are specially addressing the problems of exacerbations and are focused on their prevention. Exacerbations can cause damage to the airways and lungs, leading to a more rapid decline in lung function over time. Addressing exacerbations promptly and effectively can improve quality of life, prevent further lung damage, and reduce healthcare costs (*Wedzicha, 2004*).

Even a single COPD exacerbation can result in a significant increase in the rate of decline in lung function (*Halpin et al., 2017*; *Dransfield et al., 2017*). Frequency of exacerbations contributes to long term decline in lung function of patients with moderate to severe COPD (*Donaldson et al., 2002*). Identification and correct assessment of COPD exacerbations is paramount, given it will strongly influence therapeutic success (*Oliveira et al., 2017*).

Exacerbations are categorized into mild, moderate, and severe ones in terms of either clinical presentation (number of symptoms) or utilization of health care resources (*Wedzicha et al., 2013*). Most of the current knowledge about COPD exacerbations is based on the evidence we have for a moderate and severe exacerbations. Mild COPD exacerbations are less well studied (*Miravitlles et al., 2004*). Even mild and unreported exacerbations might negatively affect the health-related quality of life (QOL) and lung function (*Wilkinson et al., 2004*). Based on a Japanese study, there is also the phenotype of the frequent mild COPD exacerbator but there is not enough data of the impact of this phenotype on the course of the disease (*Sato et al., 2016*).

The results of our investigation showing that the question "Have you had a severe cold with cough or bronchitis during preceding 12 months?" in the MARKO questionnaire is predictive for the development of COPD in the population at risk is moving our focus even further. The question that warrants further investigation is whether those events are the "exacerbations" of biologically present but not jet clinically overt COPD?

Exacerbations of COPD are thought to be caused by complex interactions between the host, bacteria, viruses, and environmental pollution. Respiratory infections are important triggers of acute exacerbations of COPD (*Love & Proud, 2022*). Recent findings connect the frequency and severity of LRTIs prior to COPD diagnosis with increasing rates of subsequent exacerbations and increasing risk of all-cause and COPD-related mortality (*Whittaker et al., 2022*). Little is known about the association of lower respiratory tract infections before chronic obstructive pulmonary disease and future course of the disease. Based on our results, smokers prone to frequent LRTIs could be at increased risk for the progression of lung damage to clinical COPD. Further investigation should be focused on the possibility to prevent that and cut the chain of events that lead from biological COPD to clinical one.

Other important finding from our study is that there is the association between the change in EBT after a smoked cigarette (ΔEBT) and development of clinical COPD in the patients at risk. About 8 percent of middle-aged male smokers progress to moderate COPD over five years (*Geijer et al., 2006*). Since COPD progression is associated with an enhanced chronic inflammatory response in the airways and lung tissue to harmful particles or gases (*Vestbo et al., 2013*), measuring that inflammatory response could

separate the susceptible patients from the other smokers. The inflammation observed in the lungs of COPD patients appears to be a modification of the normal inflammatory response to chronic irritants such as cigarette smoke. Inflammation in respiratory diseases causes hypervascularization and increased blood flow in the airway wall with subsequent increase in the temperature of the affected tissues, and the airway temperature can be a correlate to peripheral airway inflammation (*Tufvesson et al., 2020*).

We have previously demonstrated that there is the difference in the change of EBT after the cigarette exposure (*Labor et al., 2016a*) in patients that progress to clinical COPD. Since those patients are at greater risk for COPD development, measurement of reactivity of EBT after the acute exposure to the environmental factor (cigarette smoke or other pollutants) could be the marker of the pathophysiological COPD before the lung function decline. Strict monitoring of those patients for respiratory infections/exacerbations with early prevention of the elicit exposure could prevent the further irreversible lung injury and preserve the healthy lung function and postpone or even avert the COPD development.

There are some limitations to our study. The sample size is rather small and the expected incidence of COPD was not reached. Although we have tested new simple tools as markers of future COPD some other markers (genetic, epigenetic, metabolomics) could add some additional value. Also we didn't have a control group of non-smokers of the same age and sex. This limitations were due to budget restrictions and one should consider them when evaluating the results of our study. The positive thing is that we showed that there is at least a limited power of predictability for incident COPD that lies in simple markers like EBT or a questionnaire that could be used globally for screening. Our results however have to be corroborated and validated by other comparable studies and in a broader population.

CONCLUSIONS

Chronic obstructive pulmonary disease (COPD) is one of the top causes of morbidity and mortality worldwide. At the time of diagnosis, there is already irreversible lung damage. To prevent that, we should define the parameters for detection of patients with early pathophysiological changes and initiate the necessary measures for preventing further lung damage. Based on our results, a simple self-administered questionnaire with questions about breathlessness and severe cold with cough or bronchitis during preceding months can detect early changes in smokers/ex-smokers. It seems that also the change of EBT after the cigarette exposure in smokers can detect early changes. Early interventions based on these results should be tested for efficacy in COPD prevention.

ACKNOWLEDGEMENTS

The authors wish to thank the consultant Prof. Peter MA Calverley for his valuable advice regarding study design and the construction/design and review of the MARKO questionnaire together with psychologists Professors Adrijana Košćec Đuknić and Biserka Radošević-Vidaček. The authors would also like to thank the following researchers who helped in the recruitment of participants and the data collection: Vjekoslava Amerl-Šakić,

MD; Ines Balint, MD; Merim Bezdrov, MD; Mirjana Bezdrov, MD; Ivana Boban, MD; Ljiljana Bulat Kardum, MD, PhD; Ines Diminić Lisica, MD, PhD; Albina Dumić, MD, PhD; Ljiljana Ismić, MD; Tajana Jalušić Glunčić, MD; Renata Grgurić, MD; Monika Jedud, MD; Đivo Ljubičić, MD; Tina Lukić, MD; Ljiljana Lulić-Karapetrić, MD; Suzana Maltar-Delija, MD; Dubravka Margaretić, MD; Davorka Martinković, MD; Zdenka Meštrović, MD; Nataša Mrduljaš-Đuić, MD; Jasna Nagyszombaty-Šarić, MD; Darja Nelken-Bestvina, MD; Sanja Popović Grle, MD, PhD; Sanda Pribić, MD; Jadranka Radman, MD; Rosanda Rosandić-Piasevoli, MD; Alen Stojanović, MD; Karla Tudja, MD; Neven Tudorić, MD, PhD; Bojana Vakanjac, MD; Vanja Viali, MD.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding

The study was funded by an unrestricted grant from GlaxoSmithKline (GSK eTrack number: CRT114338). Prof. Davor Plavec and Dr. Žarko Vrbica as principal investigators and the Children's Hospital Srebrnjak have for the purpose of an investigator initiated study MARKO (ClinicalTrials.gov Identifier NCT01550679) received an unrestricted grant from GlaxoSmithKline. GlaxoSmithKline has not influenced the study design or study protocol, has no ownership rights over data gathered by the study and has no influence on this and further publications arising from data gathered through this study. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures

The following grant information was disclosed by the authors:
GlaxoSmithKline: CRT114338.

Competing Interests

Plavec Davor is an Academic Editor for PeerJ. Plavec Davor is employed by Prima Nova, a private health institution. The authors declare that they do not have any other competing interests.

Author Contributions

- Žarko Vrbica conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Justinija Steiner performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Marina Labor performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Ivan Gudelj performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.

- Davor Plavec conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.

Human Ethics

The following information was supplied relating to ethical approvals (*i.e.*, approving body and any reference numbers):

The study for all investigational sites was approved by the Children's Hospital Srebrnjak Ethics Committee. The IRB approval and Consent forms were already reviewed for the already published manuscript and approved when the protocol of the study was published (http://dx.doi.org/10.1186/s12890-017-0378-6).

Data Availability

The following information was supplied regarding data availability:

The raw data are available in the Supplemental File.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.16650#supplemental-information.

REFERENCES

Celli B, Fabbri L, Criner G, Martinez FJ, Mannino D, Vogelmeier C, Montes de Oca M, Papi A, Sin DD, Han MK, Agusti A. 2022. Definition and nomenclature of chronic obstructive pulmonary disease: time for its revision. *American Journal of Respiratory and Critical Care Medicine* **206(11)**:1317–1325 DOI 10.1164/rccm.202204-0671PP.

Donaldson GC, Seemungal TA, Bhowmik A, Wedzicha JA. 2002. Relationship between exacerbation frequency and lung function decline in chronic obstructive pulmonary disease. *Thorax* **57(10)**:847–852 DOI 10.1136/thorax.57.10.847.

Dransfield MT, Kunisaki KM, Strand MJ, Anzueto A, Bhatt SP, Bowler RP, Criner GJ, Curtis JL, Hanania NA, Nath H, Putcha N, Roark SE, Wan ES, Washko GR, Wells JM, Wendt CH, Make BJ, COPD Gene Investigators. 2017. Acute exacerbations and lung function loss in smokers with and without chronic obstructive pulmonary disease. *American Journal of Respiratory and Critical Care Medicine* **195(3)**:324–330 DOI 10.1164/rccm.201605-1014OC.

Geijer RM, Sachs AP, Verheij TJ, Salomé PL, Lammers JW, Hoes AW. 2006. Incidence and determinants of moderate COPD (GOLD II) in male smokers aged 40-65 years: 5-year follow up. *The British Journal of General Practice: the Journal of the Royal College of General Practitioners* **56(530)**:656–661.

GOLD. 2023. The global strategy for diagnosis, management and prevention of COPD (updated 2023). *Available at www.goldcopd.org* (accessed 15 August 2023).

Halpin DMG, Decramer M, Celli BR, Mueller A, Metzdorf N, Tashkin DP. 2017. Effect of a single exacerbation on decline in lung function in COPD. *Respiratory Medicine* **128(5 Pt 1)**:85–91 DOI 10.1016/j.rmed.2017.04.013.

Jones PW, Harding G, Berry P, Wiklund I, Chen WH, Kline Leidy N. 2009. Development and first validation of the COPD assessment test. *European Respiratory Journal* **34(3)**:648–654 DOI 10.1183/09031936.00102509.

Jones PW, Quirk FH, Baveystock CM, Littlejohns P. 1992. A self-complete measure of health status for chronic airflow limitation. The St. George's respiratory questionnaire. *The American Review of Respiratory Disease* **145(6)**:1321–1327 DOI 10.1164/ajrccm/145.6.1321.

Koo HK, Vasilescu DM, Booth S, Hsieh A, Katsamenis OL, Fishbane N, Elliott WM, Kirby M, Lackie P, Sinclair I, Warner JA, Cooper JD, Coxson HO, Paré PD, Hogg JC, Hackett TL. 2018. Small airways disease in mild and moderate chronic obstructive pulmonary disease: a cross-sectional study. *The Lancet Respiratory Medicine* **6(8)**:591–602 DOI 10.1016/S2213-2600(18)30196-6.

Labor M, Vrbica Ž, Gudelj I, Labor S, Jurić I, Plavec D. 2016a. Exhaled breath temperature as a novel marker of future development of COPD: results of a follow-up study in smokers. *COPD* **13(6)**:741–749 DOI 10.3109/15412555.2016.1164129.

Labor M, Vrbica Ž, Gudelj I, Labor S, Plavec D. 2016b. Diagnostic accuracy of a pocket screening spirometer in diagnosing chronic obstructive pulmonary disease in general practice: a cross sectional validation study using tertiary care as a reference. *BMC Family Practice* **17(1)**:112 DOI 10.1186/s12875-016-0518-8.

Lázár Z, Bikov A, Martinovszky F, Gálffy G, Losonczy G, Horváth I. 2014. Exhaled breath temperature in patients with stable and exacerbated COPD. *Journal of Breath Research* **8(4)**:46002 DOI 10.1088/1752-7155/8/4/046002.

Love ME, Proud D. 2022. Respiratory viral and bacterial exacerbations of COPD-the role of the airway epithelium. *Cells* **11(9)**:1416 DOI 10.3390/cells11091416.

Lundbäck B, Lindberg A, Lindström M, Rönmark E, Jonsson AC, Jönsson E, Larsson LG, Andersson S, Sandström T, Larsson K, Obstructive Lung Disease in Northern Sweden Studies. 2003. Not 15 but 50% of smokers develop COPD?—report from the obstructive lung disease in northern sweden studies. *Respiratory Medicine* **97(2)**:115–122 DOI 10.1053/rmed.2003.1446.

Martinez FJ, Agusti A, Celli BR, Han MK, Allinson JP, Bhatt SP, Calverley P, Chotirmall SH, Chowdhury B, Darken P, Da Silva CA, Donaldson G, Dorinsky P, Dransfield M, Faner R, Halpin DM, Jones P, Krishnan JA, Locantore N, Martinez FD, Wedzicha JA. 2022. Treatment trials in young patients with chronic obstructive pulmonary disease and pre-chronic obstructive pulmonary disease patients: time to move forward. *American Journal of Respiratory and Critical care Medicine* **205(3)**:275–287 DOI 10.1164/rccm.202107-1663SO.

Mathers CD, Loncar D. 2006. Projections of global mortality and burden of disease from 2002 to 2030. *PLOS Medicine* **3(11)**:e442 DOI 10.1371/journal.pmed.0030442.

Miravitlles M, Ferrer M, Pont A, Zalacain R, Alvarez-Sala JL, Masa F, Verea H, Murio C, Ros F, Vidal R, IMPAC Study Group. 2004. Effect of exacerbations on quality of life in patients with chronic obstructive pulmonary disease: a 2 year follow up study. *Thorax* **59(5)**:387–395 DOI 10.1136/thx.2003.008730.

Oliveira AS, Munhá J, Bugalho A, Guimarães M, Reis G, Marques A, GI DPOC Grupo de Interesse na Doenca Pulmonar Obstrutiva Cronica. 2017. Identification and assessment of COPD exacerbations. *Pulmonology* **S2173–5115(17)**:30165–30173 DOI 10.1016/j.rppnen.2017.10.006.

Popov TA, Dunev S, Kralimarkova TZ, Kraeva S, DuBuske LM. 2007. Evaluation of a simple, potentially individual device for exhaled breath temperature measurement. *Respiratory Medicine* **101(10)**:2044–2050 DOI 10.1016/j.rmed.2007.06.005.

Popov TA, Kralimarkova TZ, Labor M, Plavec D. 2017. The added value of exhaled breath temperature in respiratory medicine. *Journal of Breath Research* **11(3)**:034001 DOI 10.1088/1752-7163/aa7801.

Quanjer PH, Stanojevic S, Cole TJ, Baur X, Hall GL, Culver BH, Enright PL, Hankinson JL, Ip MS, Zheng J, Stocks J, ERS Global Lung Function Initiative. 2012. Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. *European Respiratory Journal* **40(6)**:1324–1343 DOI 10.1183/09031936.00080312.

Roca J, Rodriguez-Roisin R, Cobo E, Burgos F, Perez J, Clausen JL. 1990. Single-breath carbon monoxide diffusing capacity prediction equations from a Mediterranean population. *American Review of Respiratory Disease* **141(4 Pt 1)**:1026–1032 DOI 10.1164/ajrccm/141.4_Pt_1.1026.

Sato M, Chubachi S, Sasaki M, Haraguchi M, Kameyama N, Tsutsumi A, Takahashi S, Nakamura H, Asano K, Betsuyaku T. 2016. Impact of mild exacerbation on COPD symptoms in a Japanese cohort. *International Journal of Chronic Obstructive Pulmonary Disease* **11**:1269–1278 DOI 10.2147/COPD.S105454.

Sin DD. 2023. The Importance of early chronic obstructive pulmonary disease: a lecture from 2022 Asian Pacific society of respirology. *Tuberculosis and Respiratory Diseases* **86(2)**:71–81 DOI 10.4046/trd.2023.0005.

Stolz D, Mkorombindo T, Schumann DM, Agusti A, Ash SY, Bafadhel M, Bai C, Chalmers JD, Criner GJ, Dharmage SC, Franssen FME, Frey U, Han M, Hansel NN, Hawkins NM, Kalhan R, Konigshoff M, Ko FW, Parekh TM, Powell P, Rutten-van Mölken M, Simpson J, Sin DD, Song Y, Suki B, Troosters T, Washko GR, Welte T, Dransfield MT. 2022. Towards the elimination of chronic obstructive pulmonary disease: a lancet commission. *Lancet* **400(10356)**:921–972 DOI 10.1016/S0140-6736(22)01273-9.

Troosters T, Gosselink R, Decramer M. 1999. Six minute walking distance in healthy elderly subjects. *European Respiratory Journal* **14(2)**:270–274 DOI 10.1034/j.1399-3003.1999.14b06.x.

Tufvesson E, Nilsson E, Popov TA, Hesselstrand R, Bjermer L. 2020. Fractional exhaled breath temperature in patients with asthma, chronic obstructive pulmonary disease, or systemic sclerosis compared to healthy controls. *European Clinical Respiratory Journal* **7(1)**:1747014 DOI 10.1080/20018525.2020.1747014.

Vestbo J, Hurd SS, Agusti AG, Jones PW, Vogelmeier C, Anzueto A, Barnes PJ, Fabbri LM, Martinez FJ, Nishimura M, Stockley RA, Sin DD, Rodriguez-Roisin R. 2013. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. *American Journal of Respiratory and Critical Care Medicine* **187(4)**:347–365 DOI 10.1164/rccm.201204-0596PP.

Vrbica Ž, Labor M, Guddj I, Labor S, Jurić I, Plavec D, MARKO Study Group. 2017. Early detection of COPD patients in GOLD 0 population: an observational non-interventional cohort study—MARKO study. *BMC Pulmonary Medicine* **17(1)**:36 DOI 10.1186/s12890-017-0378-6.

Vrbica Ž, Labor M, Košćec Đuknić A, Radošević-Vidaček B, Gudelj I, Labor S, Jurić I, Calverley PM, Plavec D. 2016. Development and the initial validation of a new self-administered questionnaire for an early detection of health status changes in smokers at risk for chronic obstructive pulmonary disease (MARKO questionnaire). *Croatian Medical Journal* **57(5)**:425–433 DOI 10.3325/cmj.2016.57.425.

Wedzicha JA. 2004. Early therapy improves outcomes of exacerbations of chronic obstructive pulmonary disease. *American Journal of Respiratory and Critical Care Medicine* **169(12)**:1298–1303 DOI 10.1164/rccm.200310-1443OC.

Wedzicha JA, Decramer M, Ficker JH, Niewoehner DE, Sandström T, Taylor AF, D'Andrea P, Arrasate C, Chen H, Banerji D. 2013. Analysis of chronic obstructive pulmonary disease exacerbations with the dual bronchodilator QVA149 compared with glycopyrronium and tiotropium (SPARK): a randomised, double-blind, paralled-group study. *The Lancet Respiratory Medicine* **1(3)**:199–209 DOI 10.1016/S2213-2600(13)70052-3.

Whittaker H, Nordon C, Rubino A, Morris T, Xu Y, De Nigris E, Müllerová H, Quint JK. 2022. Frequency and severity of respiratory infections prior to COPD diagnosis and risk of subsequent postdiagnosis COPD exacerbations and mortality: EXACOS-UK health care data study. *Thorax* **78(8)**:760–766 DOI 10.1136/thorax-2022-219039.

Wilkinson TM, Donaldson GC, Hurst JR, Seemungal TA, Wedzicha JA. 2004. Early therapy improves outcomes of exacerbations of chronic obstructive pulmonary disease. *American Journal of Respiratory and Critical Care Medicine* **169(12)**:1298–1303 DOI 10.1164/rccm.200310-1443OC.

Woodruff PG, Barr RG, Bleecker E, Christenson SA, Couper D, Curtis JL, Gouskova NA, Hansel NN, Hoffman EA, Kanner RE, Kleerup E, Lazarus SC, Martinez FJ, Paine R III, Rennard S, Tashkin DP, Han MK, SPIROMICS Research Group. 2016. Clinical Significance of symptoms in smokers with preserved pulmonary function. *New England Journal of Medicine* **374(19)**:1811–1821 DOI 10.1056/NEJMoa1505971.

4. ZNANSTVENI DOPRINOS OBJEDINJENIH RADOVA

Prikazani radovi dio su rezultata projekta MARKO (MArkeri Rane dijagnostike bolesti u ispitanika rizičnih za razvoj KOPB-a). Provođenjem MARKO protokola po prvi je puta za Hrvatsku utvrđena pojavnost KOPB-a u nedijagnosticiranih osoba iz rizične skupine (pušači/bivši pušači). Također je po prvi puta utvrđena učestalost komorbiditeta u pušača/bivših pušača različite razine simptoma te stupnja težine KOPB-a. Time je prvi puta utvrđena veličina problema u navedenoj populaciji te utvrđen temelj za daljnja istraživanja i moguće intervencije u populaciji u kojoj KOPB nije do sada dijagnosticiran, a postoji značajni rizik za razvoj istog. Rezultati ovog istraživanja omogućuju analizu naše populacije i planiranje daljih znanstvenih istraživanja i javno zdravstvenih aktivnosti prema stanju u RH. Također je ovim istraživanjem razvijen i definiran novi alat za procjenu i praćenje pušača s rizikom za razvoj KOPB-a kao i oboljelih od blažih oblika KOPB-a. Novo razvijeni MARKO upitnik je pokazao značajnu povezanost s rezultatima postojećih kompleksnijih HRQoL upitnika koji su previše kompliciran za redovitu kliničku uporabu te nedovoljno osjetljivi za otkrivanje i razlikovanje početnih oblika KOPB-a. MARKO upitnik je prvi puta pokazao da je HRQoL upitnikom moguće razlikovati rane oblike bolesti, a ispitan je u našoj populaciji. Također je utvrđeno da je primjenjiv u ambulantnim uvjetima uz mogućnost samo-ispunjavanja od strane ispitanika što omogućuje njegovu široku primjenu (probir) te time olakšava dalja znanstvena istraživanja KOPB-a. MARKO upitnik je moguće prevesti i na druge svjetske jezike. Kako je široki zahvat u praćenju rizičnih pušača i oboljelih od KOPB-a u općoj populaciji značajno ograničen smanjenom dostupnosti i relativnoj kompleksnosti spirometrije (tehnički i interpretacijski zahtjevna) kao temeljnog dijagnostičkog postupka u dijagnostici i praćenju KOPB-a, studijom je utvrđena mogućnost uporabe jednostavnog priručnog analizatora plućne funkcije (COPD6) na razini primarne zdravstvene zaštite. Utvrđeno je da je COPD6 pouzdano sredstvo u rukama liječnika obiteljske medicine te je utvrđen preporučeni algoritam njegove uporabe koji omogućuje šire praćenje populacije pod rizikom za razvoj KOPB-a. Studija je prvi puta definirala mjerenje temperature izdahnutog zraka prije i nakon udisanja duhanskog dima kao biljega aktivnosti upalnog procesa u KOPB-u. Navedena spoznaja otvara nove mogućnosti diferenciranja osjetljivih pušača i analiziranja ranih patofizioloških procesa koji dovode do oštećenja pluća u KOPB-u. Pogoršanja (egzacerbacije) KOPB-a su jedan od temelja pogoršanja bolesti. U našem ispitivanju je domena odgovora o respiracijskim infekcijama povezana s incidencijom KOPB-a u praćenoj populaciji. Navedeno je značajna novost koja upućuje na potrebu redefiniranja termina egzacerbacija KOPB-a i analizu učinka blagih egzacerbacija na progresiju

bolesti u rizičnoj populaciji. To otvara cijelo područje novih istraživanja u cilju boljeg razumijevanja patofiziologije ranih promjena u KOPB-u.

5. ŽIVOTOPIS

OSOBNI PODACI

Ime i prezime	**mr.sc. Žarko Vrbica, prim.,dr.med.**
Adresa	
Telefon	
Telefaks	
Elektronička pošta, Web adresa	
Državljanstvo	Hrvatsko
Datum rođenja	22.03.1963.
Matični broj iz Upisnika znanstvenika	**259013**

RADNO ISKUSTVO

• Datumi (od – do)	2014-
Ustanova zaposlenja	Opća bolnica Dubrovnik
Naziv radnog mjesta	Internist- pulmolog
Funkcija	Voditelj odjela za pulmologiju i imunologiju
Područje rada	Pulmologija
• Datumi (od – do)	2014-
Ustanova zaposlenja	Sveučilište u Dubrovniku
Naziv radnog mjesta	Viši Predavač
Funkcija	Sestrinski studij: Voditelj kolegija Fiziologija i Patofiziologija / Kliničko sestrinstvo: Voditelj kolegija Onkologija
Područje rada	Fiziologija, Patofiziologija, Pulmologija i Onkologija
• Datumi (od – do)	2012-2013.
Ustanova zaposlenja	Opća bolnica Dubrovnik
Naziv radnog mjesta	Internist- pulmolog
Funkcija	Voditelj internog odjela
Područje rada	Interna medicina - Pulmologija
• Datumi (od – do)	2008.-2014.
Ustanova zaposlenja	Sveučilište u Dubrovniku
Naziv radnog mjesta	Predavač
Funkcija	Sestrinski studij: Voditelj kolegija Fiziologija i Klinička medicina I
Područje rada	Fiziologija, Patofiziologija, Klinička medicina (internističke grane)
• Datumi (od – do)	2004.-2012.
Ustanova zaposlenja	Opća bolnica Dubrovnik
Naziv radnog mjesta	Internist-pulmolog
Područje rada	Pulmologija
• Datumi (od – do)	1996.-2004.
Ustanova zaposlenja	Opća bolnica Dubrovnik

Naziv radnog mjesta	Internist
Funkcija	Liječnik na odjelu
Područje rada	Interna medicina
• Datumi (od – do)	1989.-1995.
Ustanova zaposlenja	Opća bolnica Dubrovnik
Naziv radnog mjesta	Hitna pomoć
Funkcija	Liječnik hitne pomoći
Područje rada	Urgentna medicina

ŠKOLOVANJE

Datum	2003.
Mjesto	Zagreb
Ustanova	Klinika za plućne bolesti „Jordanovac“
Zvanje	Internist-pulmolog
Datum	2003.
Mjesto	Zagreb
Ustanova	Medicinski fakultet, Sveučilišta u Zagrebu
Zvanje	Magistar znanosti
Datum	1999.
Mjesto	Zagreb
Ustanova	KBC „Zagreb“
Zvanje	Internist
Datum	1988.
Mjesto	Beograd
Ustanova	Medicinski fakultet, Sveučilišta u Beogradu
Zvanje	Liječnik

USAVRŠAVANJE

Godina	2004.
Mjesto	Dubrovnik i Zagreb
Ustanova	Hrvatsko društvo za reanimatologiju
Područje	Instruktor ERC
Godina	1998.
Mjesto	Brno
Ustanova	ESPEN
Područje	Enteralna i parenteralna prehrana
Godina	1996.
Mjesto	Stubičke Toplice
Ustanova	Lebanon/Zagreb Partnership Leadership Development Program
Područje	Management u medicini

OSOBNE VJEŠTINE I KOMPETENCIJE	
Materinji jezik	Hrvatski
Strani jezici	
Jezik	Engleski
Govori	Da
Piše	Da
Čita	Da
Jezik	Talijanski
Govori	Da
Piše	Da
Čita	Da
Jezik	Češki
Govori	Da
Piše	Ne
Čita	Da
SOCIJALNE VJEŠTINE I KOMPETENCIJE	Dobre komunikacijske i edukacijske vještine i timski rad
ORGANIZACIJSKE VJEŠTINE I KOMPETENCIJE	Iskustvo u organizaciji medicinskih sastanaka
TEHNIČKE VJEŠTINE I KOMPETENCIJE	MS Office i Internet
UMJETNIČKE VJEŠTINE I KOMPETENCIJE	Niža glazbena (glasovir)
VOZAČKA DOZVOLA	B kategorija
DODATNI PODACI	Članstva: • Hrvatska liječnička komora • Hrvatsko pulmološko društvo • Hrvatsko društvo za medicinsku informatiku • Hrvatsko društvo za reanimatologiju • Europsko društvo za respiratorne bolesti • Europsko društvo za parentalnu i enteralnu prehranu • MENSA • ROTARY Autor 35 radova od čega 21 objavljen u inozemstvu

Predavač na 53 stručna skupa od čega 26 međunarodnih

Autor i koautor poglavlja u 14 knjiga

Odlukom Povjerenstva za priznavanje naziva Primarijus Ministarstva zdravstva Republike Hrvatske od 26. travnja 2017. godine priznat naziv Primarijus.

Hrvatski branitelj – dragovoljac iz Domovinskog rata