21

4242-A-2208

KOL är associerad med utbredd differentiell metylering i celler från bronkoalveolärt lavage

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Bakgrund

Kartläggning av sambandet mellan KOL och DNA-metyleringsmönster kan bidra till en bättre förståelse av sjukdomens patogenes.

Material och metod

Vi genomförde en epigenomtäckande associationsstudie (eng. EWAS) på celler från bronkoalveolärt lavage (BAL). 18 forskningspersoner med KOL och 15 kontroller (ex-rökare och rökare) genomgick bronkoskopi. DNA-metyleringsmönster undersöktes med hjälp av Illuminas MethylationEPIC BeadChip som täcker mer än 850000 CpG-siter i genomet. Differentiellt metylerade positioner (DMP:er) identifierades och analyserades utifrån 1) samband med sedan tidigare kända Pathways och genassociationer med hjälp av Kyoto Encyclopedia of Genes- and Genomes- och Gene Ontology-databaserna; 2) association med accelererat åldrande med hjälp av Horvaths epigenetiska klocka; 3) korrelation med genuttryck; och 4) samlokalisation med genetisk variation.

Resultat

1155 DMP:er uppvisade en signifikant association med KOL (Bonferroni-justerat $P < 6{,}74 \times 10\text{-}8$), många med betydande effektstorlekar. De funktionella analyserna identifierade biologiskt rimliga Pathways och genassociationer, inklusive sådana relaterade till transkriptionsfaktoraktivitet. Associationen mellan DNA-metyleringsmönster och kronologisk ålder var stark, däremot fann vi inget samband mellan KOL och accelererat åldrande. För 79 av DMP:erna korrelerade metyleringsgrad signifikant med genuttryck i BAL. 39 procent av DMP:erna låg nära enbaspolymorfier (eng. SNP) associerade med KOL.

Slutsats

Såvitt känt är detta den första epigenomtäckande associationsstudien på KOL baserad på celler från BAL, och våra analyser visade på utbredd differentiell metylering. Integration med mRNA-data identifierade relevanta gener vars uttryck tycks påverkas direkt av DNA-metyleringsgrad. Nästan hälften av DMP:erna låg nära enbaspolymorfier som sedan tidigare associerats med KOL, platser i genomet där DNA-metyleringsstatus förefaller dikteras av genetiska faktorer.

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22

4248-A-2208

The role of early warning scoring systems NEWS and MEWS in the acute exacerbation of COPD

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Background: Acute exacerbations of COPD (AECOPD) are the most devastating events in the course of the disease and one of the most common causes for in-hospital admission. Our aim was to investigate the value of early warning scoring systems NEWS and MEWS in AECOPD and examine the eventual superiority of one of the scores for this patient group.

Material and methods: This is a prospective observational study of patients with AECOPD who were admitted at hospital. The NEWS and MEWS scores were registered at admission (NEWS-1, MEWS-1) and on the second day (NEWS-2, MEWS-2). Follow-up was done at 3 and 6 months after hospitalization. A nasopharyngeal and sputum sample was taken for culture. Any possible correlations between NEWS and MEWS and other parameters of COPD were explored.

Results: A total of 64 patients were included. Mean age was 71±9 years and mean FEV1% was 36±13. In-hospital mortality was 4,7% while total mortality at 6 months was 26%. We did not find any significant correlation between in-hospital mortality and any of the scores but we could show a higher mortality rate and more frequent new acute exacerbations at 6 months of follow-up for those with higher NEWS-2. NEWS-2 was also associated with higher pCO2 at presentation and a more frequent need of NIV. Higher NEWS-1 and NEWS-2 were predictive of a longer hospital stay. The presence of pathogens in the nasopharyngeal sample was related with a higher reduction of both scores on the second day.

Conclusion: We support the superiority of NEWS as a prognostic tool in the evaluation of hospitalized patients with AECOPD in comparison to MEWS. A remaining high NEWS score at the second day of hospital stay signals a high risk of hypercapnia and need of NIV but also higher mortality and more frequent exacerbations at 6 months after AECOPD.

23

4250-A-2208

Kvarstående förändringar i surfaktantsammansättning sex månader efter svår Covid-19

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Bakgrund

SARS-CoV-2 binder till ACE2-receptorer, uttryckt på typ 2 alveolära celler, vars huvuduppgift är att producera surfaktant, som till stor del består av lipider (90%), som minskar ytspänning och deltar i immunförsvaret. Med hjälp av PExA metoden kan vi mäta förändringar i surfakantsammansättning.

Syfte och mål

För att undersöka förändringar i lipidsammansättningen av ytaktiva ämnen och förhållandet till långvariga symtom på post

covid-19 bland patienter som behandlas på intensivvårdsavdelning för covid-19-infektion.

Metoder

Patienter (n=43, 17 kvinnor, i åldern 44-80 år) som vårdats på IVA våren 2021, undersöktes vid uppföljning ca 6 månader snare med PEx, kroppspletysmografi och diffusionskapacitet för

kolmonoxid. Tjugotvå friska, ålders- och könsmatchade kontroller som ej haft Covid inkluderas som kontroll grupp.

Lipider analyserades med användning av vätskekromatografi med en trippelkvadrupol-masspektrometer. Statistiska analyser utfördes med Qlucore.

Resultat

Analyserna visar på en påverkan på surfatkantsammansättningen med signifikanta minskningar av alla uppmätta fosfatidylglyceroler (PG, n=14) en ökning av alla uppmätta fosfatidyl-inositoler (PI, n=4), t.ex. PG 18:1_18:1 22 % lägre ($p<0.001$, $q=0.04$) och PI:16:0:18:1 67 % högre ($p<0.001$, $q=0.0003$) hos personer som haft svår Covid-19 jämfört med kontroller.

Slutsats

Resultaten tyder på att surfaktatsammansättningen är påverkad lång tid efter genomgången svår Covid-19, och kan bidra till långvariga symtom från luftvägarna. Der förändade lipidmönstret liknar det man sett i djurmodeller vid fibrosutveckling.

24

4254-A-2208

Suppressive effect of corticosteroid treatment on urinary concentration of androgens and cortisol in mild and severe asthma in the cross-sectional U-BIOPRED cohort

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Background: Corticosteroids are the cornerstone in asthma treatment. However, the effects of treatment with oral corticosteroids (OCS) and inhaled corticosteroids (ICS) on the levels of endogenous steroid hormones have not been studied extensively in asthma.

Aim: To determine the influence of treatment with corticosteroids on the concentrations of endogenous steroids in urine.

Methods: Urine samples were collected from healthy controls (HC, n=99), Mild to moderate asthma (MMA; n=70) treated with low dose of ICS, Severe asthmatics (SA, n=278) treated with high dose ICS and OCS-negative, and SA treated with both high dose ICS and OCS-positive (SA; n=130). Endogenous steroids and the OCS (prednisolone) metabolites were quantified using liquid chromatography high-resolution mass spectrometry at the Swedish Doping Laboratory.

Results: Female and male urinary cortisol and androgen metabolites were significantly lower in OCS-positive SA participants than in OCS-negative SA participants (Figure 1). The most dramatic effect was in the OCS-treated SA participants compared to HC for both sexes on the levels of cortisol and dehydroepiandrosterone sulfate (DHEA-S) (Figure 1). Cortisol levels were decreased also in participants only treated with high dose inhaled corticosteroids, suggesting a dose-dependent effect on the hypothalamic–pituitary–adrenal (HPA) axis.

Conclusion: Not only oral corticosteroids but also inhaled corticosteroids dose-dependently affected both the HPA axis and the sex hormones in females and males with severe asthma. The depression of androgens in females may relate to the higher incidence of severe asthma in females because androgens are considered anti-asthmatic.

25

4255-A-2208

Functional characterisation of mast cell activation by IgE and hyperosmolarlarity in isolated human small airways

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BACKGROUND

In asthmatics, acute mast cell induced bronchoconstriction can be initiated by allergens (IgE crosslinking) and exercise (local increase of osmolarity). The aim of this study was to characterise the role of prostaglandins in the reactions by performing pharmacomechanistic studies using isolated human bronchi.

METHODS AND MATERIALS

Human small bronchi (inner diameter of 0.5-2 mm) were isolated from macroscopically healthy human lung tissue specimens obtained from patients undergoing lobectomies (n=16). The segments were incubated overnight and mounted in tissue organ baths to measure smooth muscle contractions evoked by challenge with either hyperosmolar mannitol or a monoclonal anti-human IgE antibody (anti-IgE).

RESULTS

In control segments, exposure to hyperosmolar mannitol (850 mOsm) caused an acute contraction (Emax:49±5%) reaching maximum within 10 minutes. Anti-IgE (5 µg/mL) also induced a contractile response within the same timeframe that was more powerful (Emax:89±8%). Using a combination of receptor antagonists blocking histamine H1 and cysteinyl-leukotriene cysLT1 receptors reduced both the hyperosmolar and anti-IgE induced contractions by 20% and 52%, respectively. Furthermore, by also adding a thromboxane TP receptor antagonist, both the hyperosmolar and anti-IgE induced contractions were completely prevented. Likewise, global inhibition of the cyclooxygenase (COX) enzymes and inhibition of COX-1, in combination with the H1 and cysLT1 receptor antagonism, completely prevented the bronchoconstriction for both triggers. In contrast, this effect was not observed after COX-2 inhibition, which instead enhanced the bronchoconstriction for anti-IgE by 30%, but not for mannitol.

CONCLUSIONS

It was confirmed that mast cell dependent contractions of human bronchi are mediated by histamine, cysteinyl-leukotrienes and COX-1 generated contractile prostanoids acting on the TP receptor. The relative contribution of the TP receptor was greater during hyperosmolarity than for anti-IgE challenge. Finally, the potentiation of the response to anti-IgE by COX-2 inhibition is most likely due to removal of bronchoprotective PGE2.

26

4256-A-2208

Investigating sputum periostin as a biomarker of type 2 asthma

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Background: Serum periostin associates with type-2 inflammation in asthmatic airways, but also reflects whole body periostin levels originating from multiple sources. Less is known about sputum periostin as a biomarker in asthma as detection levels are low using currently available periostin assays. We aimed to investigate detection of sputum periostin using ELISA assays targeting different periostin epitopes and relate levels to clinical characteristics.

Methods: Two ELISA systems were developed using antibodies detecting whole periostin or cleavage products, the molecular weight and amino acid sequences of which were confirmed. The ELISA assays were applied to sputum from 80 patients with mild-to-moderate and severe asthma enrolled in the European, multi-center study BIOAIR. Results were related to clinical characteristics.

Results: Sputum was found to contain smaller periostin fragments, possibly due to proteolytic cleavage at a C-terminal site. Comparing ELISA methodology using antibodies against cleaved versus whole periostin revealed detectable levels in 90% versus 44% of sputum samples respectively. Sputum periostin showed associations with blood and sputum eosinophils. Furthermore, sputum, but not serum, periostin correlated with reduced lung function, table 1.

Conclusions: We present an ELISA method for improved analysis of sputum periostin by detecting cleavage products of the periostin protein. Using this assay, sputum periostin was detectable and associated with more disease-relevant parameters in asthma than serum periostin. Sputum periostin is worth considering as a phenotype-specific biomarker in asthma as its proximity to the airways may eliminate some of the confounding factors known to affect serum periostin.

27

4257-A-2208

Does smoking status affect Type 2 inflammatory biomarkers?

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Introduction: Accurate use of biomarkers requires understanding of confounding factors. Smoking is one important life-style factor in asthma and is known to increase oxidative stress biomarkers, such as isoprostanes. We hypothesized that active smoking may influence Type 2 (T2) inflammatory biomarkers, such as FeNO (exhaled air), eosinophils and periostin in blood, as well as urinary LTE4 and PGD2 metabolites that recently have emerged as new T2 markers. Therefore, we assessed these T2 biomarkers in smokers and non-smokers in the cross-sectional study U-BIOPRED (Unbiased BIOmarkers in PREDiction of respiratory disease outcomes) study.

Methods: UBIOPRED subject data on current smoking status and presence of urinary cotinine was extracted from the eTRIKS database TranSMART, together with data from multiple T2 inflammatory biomarkers. In total, 302 severe asthmatics, non-smoking for past 12 months (having <5 pack-years), and 42 currently active smokers were included from the smoking group (n=109; at least 5 pack-years). Changes in levels of T2 biomarkers were compared by Mann-Whitney U test to define the significance and impact of self-reported current active smoking status, or when subjects were stratified by presence of urinary cotinine (n=33).

Results: FeNO was 41% lower in current active smokers ($p<0.001$). Serum periostin was 14% lower in active smokers ($p= 0.013$). In contrast, blood eosinophil counts were similar in both groups ($p=0.482$) as well as urinary LTE4 and the PGD2-metabolites (2,3-dinor-11β-PGF2a and tetranorPGDM). Stratification by presence of urinary cotinine confirmed a significant 57% decrease in FeNO and 16% in serum periostin ($p<0.041$) but confirmed no change in blood eosinophils nor urinary LTE4 and PGD2-metabolites.

Conclusions: Active smoking reduces exhaled NO (FeNO) as well as serum periostin levels in the UBIOPRED study. Blood eosinophil count and urine LTE4 and PGD2 -metabolites were not affected by active smoking.

28

4258-A-2208

Mepolizumab reduces the eosinophil activation marker eosinophil-derived neurotoxin in severe asthma patients

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Introduction: Mepolizumab blocks IL5 signalling and is used for the treatment of severe eosinophilic asthma. In randomised controlled trials mepolizumab has been shown to improve clinical endpoints, such as reduction of the number of exacerbations, but there is a great need for characterisation of therapy response in real world settings. In severe asthmatics treated with mepolizumab in the longitudinal part of BIOCROSS, [BIOmarkers in CROSS-sectional study], the levels of eosinophil-derived neurotoxin (EDN), a biomarker for eosinophil activation and degranulation, were investigated.

Methods: Plasma samples were collected from severe asthmatics before first dose and at follow-up visits at 4, 12, 24 and 36 months. EDN was measured using a research ImmunoCAP assay. The effect of mepolizumab on EDN was analysed using the Friedmans test where correction for multiple comparisons was done using Dunn´s test. A control group consisted of healthy volunteers. All results are reported as median and interquartile range.

Results: Plasma concentrations of EDN in patients before start of mepolizumab treatment were 35.2 (22.3–52.9) ng/mL (N=69). At 4 months EDN was reduced by 66% (50% – 74%) ($p < 0.0001$, N=69), and at 12 months by 67% (48% – 78%) ($p < 0.0001$; N=54), at 24 months by 66% (50% – 77%) ($p < 0.0001$; N=40) and at 36 months by 70% (56% – 78%) ($p < 0.0001$; N=18) (Figure 1). At 12, 24 and 36 months, respectively, EDN concentrations were stabilised at 11.9 (9.5-16.3) ng/mL, 12.4 (10.3-16.5) ng/mL and 12.4 (11.2-15.2). Plasma EDN concentrations in healthy volunteers were stable over 4 months: 20.1 (15.1-25.4) ng/mL (t=0) versus 18.8 (14.1-25.5) ng/mL (N=15) (t=1).

Conclusions: Mepolizumab lowers plasma concentrations of EDN, a biomarker of eosinophil activation and degranulation, in severe asthma patients.

Figure 1. Treatment with mepolizumab reduces plasma EDN in patients with severe asthma.

29

4259-A-2208

Impact of COVID-19 pandemic on BIOCROSS [BIOmarkers in CROSS-sectional] study

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Background:

The World Health Organization declared the COVID-19 outbreak a pandemic on 11 March 2020. The pandemic has had serious impact on the daily lives and healthcare of people around the world. Immediately after March 11, a decision was made to temporarily suspend all clinical research except COVID-19 related.

The aim was to illustrate how the pandemic has affected an ongoing BIOCROSS [BIOmarkers in CROSS-sectional] study at Lung and Allergy research unit at Karolinska Institutet.

Material and methods:

The BIOCROSS study is an investigation of constitutive and disease-related factors on basal levels of non-invasive biomarkers. The study includes asthmatics, participants with COPD and other respiratory diseases, and healthy controls for comparison. Severe asthmatics treated with biologics are followed longitudinally up to 3 years for assessment of response. In this analysis, we included all visits for all participants (Figure 1).

Results: Due to the pandemic, recruitment of subjects into the study was paused and no visits were carried out from mid-March 2020 (Figure 1). Virus protection equipment was needed for medical care. The staff at the Lung-Allergy reception took care of covid-sick patients. Most patients had video contact instead of physical, where possible. Research staff met severe asthmatics who needed to continue with the injection of biologic on clinical indication regardless of whether they were included in the study or not. Control of the patient was done according to asthma control questionnaires, as spirometry and FeNO control could not be performed due to risk of infection. Visits were later resumed in August 2020.

Conclusion: The COVID-19 pandemic, particularly the first wave, has had significant effects on the BIOCROSS study. In some cases, the intervals between visits were longer than planned and, in some cases, this led to missed data. Recruitment to the study was delayed, which will require extra financial resources.