Concept sheet for publication

High or low. Nasal Nitric Oxide across mutations in Primary Ciliary Dyskinesia. A Genotype/phenotype analysis of nasal NO in patients with PCD within the European reference network (ERN)

Writing team

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Possible data providers

- All ERN (European reference network) - Lung members and affiliating or supporting partners
- All members of BEAT-PCD and BESTCILIA

Authorship suggestion

Data providers and departments involved in the study will be offered a co-authorship according to the **criteria of the International Committee of Medical Journal Editors**, which include all the following: i) substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; ii) drafting the work or revising it critically for important intellectual content; iii) final approval of the version to be published; and iv) agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Authors are suggested as June K Marthin and Johanna Raidt as shared first authors and Eric G Haarman and Heymut Omran as shared last authors. Kim G Nielsen as second last author. Other authors in alphabetic order. Trial sites are offered a maximum of four co-authorships (1: > 10 PCD patients; 2: > 30 PCD patients; 3: > 60 PCD patients; 4: > 100 patients) among investigators.

All other partners and collaborators contributing to this trial shall be duly acknowledged in the publication.

This effort is supposed to result in a joint international publication with all participating centers, but importantly each center or national register retains the possibility and rights to publish the results separately and independently of the overall publication.

Background

Nasal Nitric Oxide (nNO) concentration is usually low or very low in patients with Primary Ciliary Dyskinesia (PCD) for yet unknown reasons (1).

Measured nNO holds a strong ability to separate healthy subjects from patients with PCD in both childhood and adulthood and across several different nNO sampling modalities (2) (3–5) and nNO is widely used as an important supplementary diagnostic test for PCD work up in both Europe (6) and North America (7).

Low nNO in PCD was first reported 26 years ago (8). Nasal NO has been associated with host paranasal sinus defense as sufficient nNO production in non-PCD subjects is thought to play a role in maintaining paranasal sinus sterility (9). Furhermore, ciliary beating seems to be upregulated by a NO dependent pathway in bovine airway epithelium (10), influencing mucociliary clearance. However, human in vitro studies of ciliated airway cells in air-liquid-interface (ALI) culture have been ambiguous as to whether the biosynthesis of NO in PCD is impaired (11) (12) or not (13; 14) and the etiology of low nNO in PCD and presumed link to ciliary beating remains unclear.

So far, attempts to link PCD phenotype and genotype has indicated that patients with PCD harboring CCDC39 and CCDC40 mutations may have a poorer lung function development (15).

In rare cases of PCD (<5%) (16) nNO concentration is within normal range. More than 14 different PCD-causing genes (e.g *RSPH1, GAS8, RPGR, CCNO, CCDC103, CFAP221, DNAH9, FOXJ1, GAS2L2, LRRC56, NEK10, SPEF2, STK36, TTC12*) has been associated with nNO values above the agreed cut off for nNO-production rate of 77 nL/min in a few patients with PCD (16). However, individuals with NEK10 or FOXJ1 mutations, for example, display a very severe respiratory phenotype (17), but making a diagnosis is challenging because of normal nNO values as well as apparently normal ciliary beating.
Since nNO also holds potential as an outcome parameter in future clinical trials of PCD, better understanding of nNO in PCD is warranted.

Demand of large number of patients with PCD is crucial, keeping the rareness of near-normal and normal nNO levels in PCD in mind. Motivated by the analysis of lung function in a large cohort of genotyped PCD-patients, this multicenter involvement

across international PCD centers is an obvious opportunity for gaining such further knowledge with the focus on nasal NO.

Aims:

The aims of this paper are:

1. Correlation between nasal NO levels and distinct PCD genotypes
2. Determination of further parameters potentially associated with nasal NO levels in genotyped PCD individuals
    a. course of clinical manifestations (e.g. neonatal distress, infections, bronchiectasis)
    b. diagnostic results (HVMA, TEM, IF)
    c. lung function outcome (FVC, FEV1)

Methods

Inclusion criteria:

1. Patients with a genetically confirmed diagnosis of PCD (bi-allelic mutations in a gene, known to cause PCD) with typical clinical symptoms of PCD
2. PCD individuals of all age groups with at least one nNO measurement performed according to diagnostic guidelines. Serial nNO measurements should be included if available (e.g. yearly), at least for infants and young children

Possible additional data:

1. Cross-sectional or longitudinal spirometry measurements
    a. FEV1 and FVC with date and height at the performed measurement
    b. 3-4 different spirometry measurements in at least 2 years of follow up iv available
2. Further diagnostic results such as HVMA, TEM and/or IF
3. Clinical characteristics such as situs abnormalities, congenital heart disease, infections/exacerbation, *Pseudomonas aeruginosa*, sinonasal or thoracic surgery

Data collection and preparation:

In agreement with the participating centres, we will use the dataset already generated for the “International study on genotype/phenotype correlation with focus on lung function in patients with Primary Ciliary Dyskinesia (PCD)”. Participating centres will have to provide nasal NO measurements and other potentially missing items for already entered individuals or whole datasets in case of entering individuals which were not part of the lung function study mentioned above. Additional data might be requested by study coordinators.

Genotype of patients should including information of the exact mutations by indicating the disease specific gene including both DNA- and protein-level (e.g. *DNAH5* (Exon 20: c.3036_3041delAGCG, p.V1014Lfs*20 het. + Exon 25: c.C3949T, p.Q1317* het.)). Results of segregation of autosomal recessive mutations should be provided if available.

Assessment of Nasal NO concentration measured by a NO chemiluminescence analyzer. Mean nNO concentration (ppb) from tripple measurements will be used and from those values the nNO production rate (nL/min) will be calculated using the following formula:

NO production rate (nL/min) = NO concentration (ppb) x standard flow rate (L/min).

Velum closure technique should be used as the preferred nNO sampling technique according to existing guidelines (6) (18) but tidal breathing (non-velum closure) technique is accepted in individuals not able to perform velum closure. It needs to be clearly specified whether velum closure or non-velum closure sampling has been used.

Online and off line nNO measurements: both online and off-line measurements can be included in the study but it needs to be clearly specified which of these two sampling modalities that has been used, since the nNO values may differ significantly (19).

Ethical Considerations, Data handling and safety

All participating centres have to ensure to have appropriate Ethics Approvals for their institution in accordance with national governing bodies. All data shall only be provided strictly anonymized and following applicable national/local data protection laws.

Datasets generated for this study will be handled standardized according to ERN-LUNG data safety measures.

Target journal(s):

- American Journal of Respiratory and Critical Care Medicine and Lancet RM
- Other possibilities: Thorax, European Respiratory Journal, Chest

Anticipated Milestones:

- Collaborators consent this concept sheet until 30.09.2020
- All partners deliver datasets until 30.11.2020
- Data cleaning and analysis until 31.03.2021
- Circulating a first draft of the manuscript by 31.05.2021

Literature

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