



ECFS Diagnostic Network Working Group

(Coordinator: Nico Derichs, Berlin)

Meeting Report

11th Annual Meeting, Berlin, 13-15th February 2014

Hörsaalruine, Medical History Museum, Charité University Berlin



ECFS ICM SOP & ECFS NPD SOP Training Workshops

On 12 and 13 Feb 2014, as pre-meetings, Hands-on Training Workshops for ECFS ICM SOP and ECFS NPD SOP were conducted, organized by Isabelle Sermet, Inez Bronsveld and Nico Derichs. 30 participants from 13 countries were actively present in the workshops and made a great progress on further standardization of both techniques within the European Network, to support the multicentre network standardization for these CFTR biomarkers.

Welcome and Opening

The 11th Annual DNWG meeting was then opened by Annette Grüters-Kieslich, Dekanin of Charité Universitätsmedizin Berlin and Doris Staab, Head of CF Centre Charité Berlin. The meeting was attended by 70 participants from 20 countries in Europe, USA, South America and Australia. Representatives from CFF, ECFS CTN, ECFS Patient Registry, CF Europe, German CF Association and industry were present and created an interactive forum of excellent networking.

Structures for implementation of CF Newborn Screening (Chair: Kevin Southern, Liverpool)

Jürg Barben, St. Gallen, talked about structures in existing European CF newborn screening programmes. He gave a survey of how different items are organized and reported on the Swiss NBS algorithm and results.

Jutta Hammermann from Dresden and Olaf Sommerburg from Heidelberg gave a talk on implications from German regional CF NBS experiences. Dr Hammermann pointed out the difficulty of NBS because of the great national genetic diversity in Germany and the IRT/PAP concept as a good solution conducted in Saxony. Dr Sommerburg presented an overview of strategies from the 2 German sites using the IRT-PAP method and concluded that the protocol could be used for NBS without DNA analysis and had a lower rate of detected healthy carriers. He suggested some changes in the cut-offs to elevate the sensitivity.

Afterwards, Manfred Ballmann, Bochum, presented more aspects of CF NBS in Germany. He stated that a nationwide CF NBS in Germany was more likely than ever, but still settled. Thereby he pointed out as main problem the gene diagnostic act.

Then Andreas Reimann, Bonn, continued on the socio-economical and political aspects of CF NBS. He illustrated cost analyses and their limitations, but also the long process of implementing NBS.

Current practice and challenges of diagnosing CF in Europe

(Chairs: Andreas Reimann, Mukoviszidose Institute Germany; Snezana Bojcin, CF Europe)

In this session, new countries introduced themselves to the Diagnostic Network Working Group and reported on their experiences with CF diagnosis and clinical care:

Ana Kotnik Pirs, Ljubljana, gave an insight on CF care and neonatal screening in Slovenia.

Rouzha Pancheva, Varna, presented epidemiologic data on CF in Bulgaria and also mentioned steps ahead in CF care.

Tiina Kahre, Tartu reported about CF care and diagnosis in Estonia. She pointed out that the age at diagnosis was still too high and named future challenges such as implementing a new sweat test method and NPD and starting a NBS.

Data from Ukraine were presented by Halyna Makukh from Lviv. She pointed out the high discrepancy between the number of patients registered and the number expected by incidence. Genetic diagnosis by a panel had been eased by the discovery of the frequent mutation 2184insA, a typical Western Ukraine mutation. She also reported about CFTR mutation analyses performed in asymptomatic azoospermic males and the results that indicated a high rate of CFTR-related disorders in the Ukraine. She emphasized

challenges as setting up an adult CF-center, creating a national CF programme and also the financing of CF drugs.

Then, Elena Amelina, Moscow, talked about CF care and challenges of diagnosis in Russia. She showed results from NBS and stated as main problems a national patient registry, a lack of central funding for genetic analysis and the lack of CF-centers in some regions.

At the end of the session, Inez Bronsveld, Utrecht, introduced the DNWG Diagnostic Webforum, which is supposed to be a diagnostic consultancy service for all ECFS members and aims to give an overview of difficult diagnostic cases and then define the needs for diagnostic improvements. Data will also be collected in a database. Dr Bronsveld also demonstrated how to send in cases.

CF Diagnosis: From Terminology to Registries (Chair: Kris De Boeck, Leuven)

The 2nd day of the meeting started with Bruce Marshall, Bethesda, who gave the keynote lecture on CF diagnosis in the US CF patient registry. He underlined that some patients in the registry did not have CF because of the difficult and at times inconclusive diagnosis as well as missing data relevant for diagnosis in the registry.

Directly afterwards, Edward McKone, Dublin, spoke about CF diagnosis in the European CF patient registry. He also named the difficult diagnosis because of the numerous CFTR mutations with varying consequences, which results in patients in the registry that do not fulfill the diagnostic criteria as elevated sweat chloride and 2 CF causing mutations. Another problem was missing data in the registry concerning diagnostic criteria. He concluded by recommending to stratify the results by mutation classes as practiced in other registries.

Then the first *Young Investigator* Michaela Kleinschmidt, Brussels, introduced the DNWG Registry project, which aims to connect the work of the DNWG and the ECFS Patient Registry and to harmonize terminology and diagnostic criteria of CF diagnosis on a European level in order to improve existing registry quality control systems. The project anticipates a discussion with ECFS Patient Registry Definition Group, regular reevaluation of documentation of CF diagnosis and involving more European countries.

Subsequently Nico Derichs, Berlin, gave an update on the European CF diagnostic guideline. He reviewed on the pathomechanisms of CFTR mutations, diagnostic guidelines and the resulting terminology such as 'CFTR-related disorder', 'CFTR dysfunction' and the newly created 'CFSPID' (CF Screened Positive, Inconclusive diagnosis) category. An update of the European CF Diagnostic Guideline is planned for 2015.

CFTR Function: Sweat gland & Intestine (Chair: Nico Derichs, Berlin)

In the session on CFTR function in sweat gland and intestine, Lutz Nährlich, Giessen, reported on new perspectives for sweat test performance. He concluded that although sweat test was the diagnostic standard, it is still a challenge, especially in NBS, and added that new tests have to prove themselves concerning practicability, sensibility and specificity.

The second *Young Investigator* Lea Pinders-Kessler, Berlin, presented results from the ECFS ICM SOP multicenter validation and reference data for CF diagnosis. Three centers participated in the validation study, and multicentre reproducibility was successfully demonstrated. Still, the Derichs ICM score was confirmed as best diagnostic cut-off for all sites, with the addition of Carbachol pre washout in patients with high residual function. Hence, the ECFS ICM SOP should be implemented more strongly in diagnostic algorithms, and ICM would also be possible in future multicenter trials.

Nico Derichs, Berlin, then tied in with ICM certification for clinical trials. He explained that centers could apply for certification at any time and would need to send in measurements from 5 known PI-CF patients with 2 known CF causing mutations and 5 healthy controls. The aim is to have 9 certified centers at the end of 2014. Additionally, the existing structure of a ICM central reading facility was introduced. Perspectively,

there should be more phase 2 trials investigating organ-specific drug responsiveness, which will most likely be performed as optional substudies.

Then, Jeffrey Beekman, Utrecht, gave a lecture on intestinal organoids and CF diagnosis. He explained the principle of creating intestinal organoids and the measurement of CFTR function by analyzing the Forskolin induced swelling and luminal area. Both assays combined gave a large diagnostic range to study individual CFTR function for diagnostic purposes as well as for CFTR modulator therapy. He concluded that reference values could be generated using organoids expressing all types of CFTR mutations and that intestinal organoids might be implicated for diagnosis, prognosis and treatment.

CFTR Function: Respiratory (Chair: Michael Wilschanski, Jerusalem)

Firstly in the next session on CFTR function in respiratory tract, Izabela Sad, Rio de Janeiro, talked about NPD experiences from Brazil. NPD is only performed in 2 Brazilian CF centers so far. They use a modified NPD method by Dr Teresinha Leal, using 4 solutions (no ATP), with a patient lying horizontally and swallowing the solutions that are applied through urinary catheters. In total they have performed 181 examinations and are going to further analyze the results. Izabela was now trained in Berlin for the ECFS NPD SOP and will introduce it in Rio de Janeiro to finally certify NPD in Brazil and participate in clinical trials.

The third *Young Investigator*, Gloria Tridello, Verona, talked about the search for NPD outcomes to support CF diagnosis. Two groups were compared: the reference population consisting of CF patients and healthy controls, and the uncertain diagnostic population. The best characteristics to detect CF were the Wilschanski Index and a modified Sermet Score. She concluded that NPD is highly significant in uncertain diagnosis.

Afterwards, the fourth *Young Investigator*, Mike Waller, London, referred about the interpretation of NPD in difficult cases and the role of published equations. The results of the retrospective analysis were that both equations work well in non CF and classic CF patients, whereas there was poor concordance between both equations in patients of diagnostic uncertainty, indicating that there is still no gold standard for analyzing NPD.

Inez Bronsveld, Utrecht, introduced the ECFS NPD SOP multicenter validation study to determine centre-independent reference values and give a standardized guideline on how to analyse and interpret NPD recordings. Centers are asked to send in 10 non-CF, 10 PI-CF and 5 PS-CF NPD measurements to participate. There are already 12 centers participating. The study aims to be finished in June 2014.

Then, Isabelle Sermet, Paris, talked about the CTN TDN NPD SOP for clinical trials. Centers need to be certified by sending in NPD tracings, as 8 centers have already done, to be qualified for interventional clinical trials using NPD as an endpoint. There will also be NPD training for comparable conditions in clinical trials. The last issue that needs to be solved is to set up a central reading CTN NPD tracings.

Multiple Breath Washout (Chair: Jane Davies, London)

The next session was about multiple breath washout. Philipp Latzin, Basel, referred about background, techniques and implementation in routine CF care. MBW is an important method to detect early lung disease in CF, because in contrast to FEV1 the small airways can be investigated mainly, which are also the first affected part of the lung. Additionally, MBW easy to learn, easy to apply and reproducible.

Subsequently, Jane Davies, London, spoke about challenges for clinical trials of Lung Clearance Index and introduced the CTN TDN MBW SOP for clinical trials. London will serve as a MBW central reading facility for the CTN.

CFTR Genetics (Chair: Burkhard Tümmler, Hannover)

The third day of the meeting started with a session on CFTR genetics. Burkhard Tümmler, Hannover, gave a lecture about how to better classify CFTR mutations in vitro into mutation classes. He concluded that in vitro

methods were very useful to uncover novel mechanisms of the molecular pathology of mutations, but they would never cover the diversity of real life, so people should focus on in vivo studies.

Afterwards, there was a “Pro/Con discussion” on CFTR2 and whether defining the most common CFTR mutations would help to predict the individual clinical phenotype. Anne Munck, Paris was arguing on the pro side. She stated that CFTR2 helped establishing CF diagnosis, CF care management and genetic counselling, although sweat test remained the most reliable tool for CF diagnosis. Kevin Southern, Liverpool, kindly agreed to argue that CFTR2 was useless predicting individual outcome. He presented a case of an 18-year-old girl with a clear diagnosis who has only a few symptoms without therapy. By that he underlined the natural variation.

Then, Sheila Scheinert, Berlin, presented a new DNWG project, CFTR3. This project aims to characterize rare CFTR mutations on a personalized level (by NPD/ICM and clinical data) and therefore synergistically adds to CFTR2. The idea is to create an open-access database with information on phenotype and CFTR function in biomarkers. The project is going to start soon, and all CF centers are invited to participate.

The fifth *Young Investigator*, Aurelie Hatton, Paris, presented data of 2 patients with stop mutations that gave insight in the pathomechanism of CF disease. She assumes that the amount of residual function influences the phenotype and depends on the position of the stop codon.

The last talk in the genetic session was given by Aleksandra Norek, Berlin, on Next Generation Sequencing in CF diagnosis. She pointed out advantages as lower cost, faster sequencing and sequencing of introns and exons, but also touched the problem of finding more variants with unknown consequence.

Translational Use of CF Diagnostic Tools (Chair: Inez Bronsveld, Utrecht)

As first speaker in the last session of the meeting, Silvia Gartner, Barcelona, referred on follow-up of equivocal cases after CF NBS in Spain. Most of the infants with inconclusive diagnosis after NBS remained healthy, but still some developed CF. She concluded that NPD or ICM might give useful additional information for a clear diagnosis, and that these infants should be followed up in a CF-center and included in CF registries.

The last *Young Investigator*, Bente Aalbers, Utrecht, gave an update on the 5T project. To date, centers from 6 countries have joined and sent in data. The results indicate that NPD helps to predict the severity in 5T patients to some extent. Also, the number of TG repeats seems to correlate with the severity of disease. Still, to date there is no recommendation for follow-up possible.

Hugo de Jonge, Rotterdam, talked about CFTR repair by genistein and curcumin in CF patients carrying the S1251 gating mutation, the Dutch equivalent for G551D. He introduced a clinical trial that is about to start to demonstrate the effect in vivo, after it has already been proved ex vivo in ICM and intestinal organoids.

Then Peter Middleton, Sydney, gave an overview on CF research in Australia. He talked about different ASL compositions that influence NPD results. Atrovent influenced NPD results by decreasing basal PD and CI-response. Hypertonic saline solutions also decreased nasal PD, whereas Mannitol does not change nasal PD. He supposed there was a link between CFTR and ENaC.

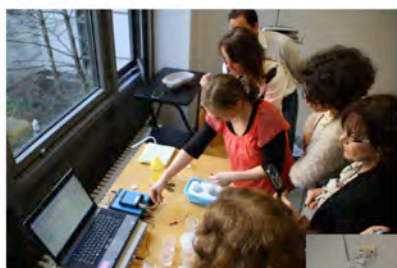
Finally, Nico Derichs closed the meeting by thanking all the participants, especially the new countries and the keynote speaker Bruce Marshall, as well as the supporting companies and bodies. He also showed the DNWG perspectives with ongoing projects like the ICM SOP, NPD SOP and 5T NPD projects, and new ones like CFTR3, Registry Project and the Webforum. Our next meeting will be on 13th June at 13.30 at the ECFC in Gothenburg. The 12th annual DNWG meeting will take place Feb 2015 in Warsaw, Poland.

Impressions from the ECFS DNWG Meeting Berlin 2014:

Berlin 2014: ICM SOP Workshop



Berlin 2014: NPD SOP Workshop





CF Newborn Screening Discussion Panel



You never walk alone...





Berlin 2014: Young Investigators



Left to right:
Gloria Tridello (Verona), Michaela Kleinschmidt (Brussels), Mike Waller (London),
Lea Pinders-Kessler (Berlin), Aurelie Hatton (Paris). Not shown: Bente Aalbers (Utrecht).



Berlin 2014



Berlin 2014





Thank you!



Local organising team:

Sheila Scheinert
Lea Pinders
Aleksandra Norek
Michaela Kleinschmidt
Izabela Sad
Kimberley Farmer

CHARITÉ UNIVERSITÄTSMEDIZIN BERLIN



Thank you!



The meeting was kindly supported by:



(7500 €)



(5000 €)



(5000 €)



(4000 US \$)



(1500 €)