

Report on the Workshop “Infections with Nontuberculous Mycobacteria”

Location: Freiburg, Germany

Date: 5th/6th November

For the Protocol: N. Töpfner, Berit Lange, Dirk Wagner

The first meeting of the NTM-NET was held on November 5th and 6th 2009 at the Universitätsklinik Freiburg in Freiburg following an invitation by **Alexandra Nieters, Philipp Henneke** and **Dirk Wagner**.

Thursday 5th of November

An introduction to the meeting was given by **Philipp Henneke** (Freiburg, GER). He explained the history of the NTM-initiative within the newly founded Centre of Chronic Immunodeficiency (CCI) in Freiburg, which organized, supported and hosted the meeting. He pointed out the motivation to organize a scientific NTM-workshop, e.g. the need for collaboration and networking in research on rare diseases like NTM-infections, and explained the aim of the NTM-NET, e.g. to facilitate collaborative research projects for childhood and adult NTM-infections in Europe by attracting all scientists and clinicians, e.g. pneumologists, adult and pediatric ID specialists, microbiologists, epidemiologists, involved or interested in NTM-research, primarily in Europe. He also explained the structure of the following meeting and explicitly asked for active participation and collection of research ideas that were going to be discussed at the end of the meeting. A welcome address was kindly held by **Stephan Ehl** (Freiburg, GER), scientific director of the Centre of Chronic Immunodeficiency in Freiburg. He explained structure and infrastructure of the newly founded CCI.

The following part of the meeting on was chaired by **Alexandra Nieters** (Freiburg, GER) and **Philipp Henneke**.

- **Jakko van Ingen** (RIVM, Bilthoven, NL) presented an overview of the extensive research conducted in the course of his PhD thesis and specifically talked about Systemic NTM-infections. He explained predisposing conditions for NTM and brought up the question why different strains of mycobacteria predominate in different predisposing conditions. He also posed the question why different mycobacteria affect the human in very different ways.
- In his talk on NTM bacteriology **Dick van Soolingen** (RIVM, Bilthoven, NL) explained in detail the clinical relevance of different NTM strains. He presented data from the RIVM lab showing a steady increasing frequency of NTM in contrast to TB-isolates since 2002. Possible reasons for this finding may be related to improved diagnostics, heightened awareness of physicians, changes related to host immunity or to – less likely - NTM pathogenicity. A combination of these factors is most likely.
- **Dirk Wagner** (Freiburg, GER) addressed in his talk on Pulmonary NTM-Infections different questions related to the epidemiological trends, the exposure of pulmonary NTM-patients, problems with the definition of „Pulmonary NTM-infections“, different clinical manifestations and predisposition to the disease. He continued with an introduction of the species involved, their differences in pathogenicity and questions related to diagnostics of species specificity and potential for diagnostic improvements. He suggested several putative study proposals to be discussed.

The next session on NTM diagnostic was chaired by **Dick van Soolingen** and **Georg Alexander Häcker** (Freiburg, GER).

- **Elvira Richter** (National Reference Center for Mycobacteria, GER) talked about Microbiological diagnostics of NTM. She focused her talk specifically on new molecular methods to identify NTM organisms and raised the question to what sublevel identification of mycobacteria was clinically relevant. Although molecular methods are widely available, she underlined that cultural isolation of mycobacteria is still 'gold standard' in diagnostics and should always be aimed for. Furthermore drug susceptibility testing was discussed and how it corresponds to in vivo resistance experience. In the discussion following her talk the point of utility of drug susceptibility testing was raised.
- **Marc Govaerts** (Brussels, BEL) from Veterinary and Agrochemical Research Centre CODA/CERVA, talked about Protein based diagnostics of NTM-infections. He focused on his findings concerning diagnostic methods for *Mycobacterium paratuberculosis* in cattle where conventional fecal cultures methods take 12-16 weeks to diagnose the infection. Thus indirect tests are highly desirable. The currently available antibody and CMI based tests have very limited specificity and sensitivity. Marc presented data on CMI based ELISA that uses *M. paratuberculosis* specific antigen with high specificity and similar sensitivity.
- **Klaus Magdorf** (Charité, Berlin, GER) kindly presented some of his data regarding diagnosis of NTM infection via IGRAs and TST in children.

During the following session - chaired by **Andrew Cant** (Newcastle, GB) and **Dirk Wagner** - Existing cohorts of NTM patients were presented.

- Unfortunately **Walter Haas** (Robert Koch Institute, Berlin, GER) had to cancel his attendance at short notice due to the developments in novel Influenza (H1N1). He kindly forwarded his data to the organization committee to be presented on his behalf. In a prospective study using a capture-recapture method in Germany, with 370 participating pediatric hospitals from 2003 to 2005 an incidence rate for NTM infection in children of 1.24/100.000 was estimated.
- **Esther van de Vosse** (Leiden, NL) presented her data on a prospective 4 year nationwide NTM lymphadenitis cohort. In total 210 patients were included of who 75 had other infections and 135 NTM infection. The predominant infections were *M. avium* and *M. haemophilum*. Of the 135 patients, 100 were randomized to either receive surgical or antibiotic therapy. In the surgical treatment group 48 were cured compared to 33 in the antibiotic treatment group. In this patient group no definite known MSDM mutation played a role. She also proposed dentition as one risk factor for NTM lymphadenitis in small children. Interestingly, two children in her cohort were autistic, who more frequently have the habit to eat soil. This could be one question to be included in a prospective epidemiological study.
- The next talk was given by **Francois-Xavier Lescure** (Paris, FR) about *M. xenopi* pulmonary infections in France and NTM pulmonary infections in Denmark. He presented one cohort of 136 patients from the east of France. Of these 91% had comorbidities and it was possible to distinguish 3 radiological forms (cavitary, nodular, infiltrative). In another prospective study from Denmark, 1282 patients were recruited. Of those, 335 had definite infection measured by ATS criteria. In a Cox regression *M. xenopi* was associated with a more severe prognosis, but not exclusively in infected patients, but also in patients considered to be colonized according to ATS criteria.
- In a next talk **Vera Katalinic-Jankovic** (Zagreb, Croatia), presented data showing even in Croatia a slow decline of TB notification rates, whereas NTM infections are increasing. Predominantly *M. avium* and *M. xenopi* were isolated. She raised the question of mechanisms of NTM drug resistance to conventional TB drugs.
- As last talk of the day **Heather Milburn** (London, GB) presented her data of NTM patients in South East London. In total retrospectively 211 cases were identified, of which

45 were excluded due to HIV infection, 87 because they failed to meet micro criteria and 22 because of missing clinical record. Patients were treated with varying courses of antibiotics. In general, the data showed that outcome for patients with *M. kansasii* is generally good, whereas outcome of patients with *M. avium*, *M. xenopi* or *M. malmoense* is less favourable.

Friday 6th of November

The first session of the day on Immunity to NTM was chaired by **Klaus Magdorf** and **Reinhard Berner** (Freiburg, GER).

- In his keynote lecture about NTM infections in Immunodeficiency **Andrew Cant** gave a very comprehensive overview of different immune defects resulting in a heightened susceptibility to NTM disease. He explained which immune defects predispose for NTM infection and talked in detail about SCID as well as CD40 Ligand deficiency and NTM infection. He also introduced MSMD and IFN-gamma/ IL12 pathway defects, which were further elucidated by the next speaker..
- **Esther van de Vosse** kindly agreed to replace **Jaap van Dissel** (Leiden, NL) who could not attend the meeting on short notice. Esther modified his talk on The role of interferons in the pathogenesis and diagnostics of mycobacterial infections and explained her center's strategy in ascertaining MSMD in patients and described different mortality rates in patients with different IFN- γ /IL12 pathway defects. She also detailed her findings of successful exon skipping of *IL12RB1* in vitro. In the following discussion Esther speculated on the suspected incidence of MSMD in patients from The Netherlands and explained her differential diagnostic procedure.
- The next talk was given by **Rui Appelberg** (Porto, Portugal), who presented the Pathogenesis of *M. avium* Infection. In particular he reported on the virulence and the killing of *M. avium* and highlighted the notion that different *M. avium*-subtypes have different pathogenicity. In the following discussion the question was raised whether cathelicidin may be an important pathogenicity factor in humans although studies suggest its unimportance in mouse models. The differences in pathogenicity of different *M. avium* strains lead to problems in the comparability of research data. Therefore, Rui Appelberg suggested to focus on certain strains in different research laboratories.

During the next session, chaired by **Dirk Wagner** and **Philipp Henneke**, the European Network on NTM-infections (NTM-NET) was discussed.

- **Christoph Lange** (Borstel, GER), chair of the TB-NET (www.tb-net.org), introduced idea, structure, success and history of the TB-NET that was founded in 2006. During the yearly TB-NET meeting in Vienna, in September 2009, the idea of the NTM-NET as well as a proposed epidemiological study was presented by **Dirk Wagner** and **Alexandra Nieters**. During this meeting the TB-NET accepted the proposal of Dirk Wagner to have an additional steering committee member representing NTM-infections and thus also accepted the NTM-NET as a new branch of the TB-NET. In the meantime the members of the TB-NET had –by mailvote- elected Dirk Wagner out of four candidates as the new steering committee member of the TB-NET representing NTM-infections for the next three years. Christoph Lange then asked for a vote by the participants of the meeting to confirm the affiliation of the NTM-NET with the TB-NET. This vote was deferred to the end of this session. The discussion focused on the necessity for a safe data storage. Dr. Lange affirmed that by reasoning of the TB-NET data is always owned by those people that enter it, even if stored in a central database. He also emphasized the need for trusting all relevant parties in an organization such as the TB-NET since otherwise a research collaboration that is based on trust would fast disintegrate.

- **Magnus Gees** and **Alexander Stephan Affeldt** (Freiburg, GER) then talked about funding possibilities for an endeavour such as the NTM-NET. In particular the 7th European Research Framework Program was introduced as a promising funding source, especially the Work Program Health 2.3.3.
- At the end of the session Phillip Henneke asked for a vote on the question of affiliation of the NTM-NET within the TB-NET. It was agreed that those persons present at the meeting would be the founding group of the NTM-NET and thus representative. The question whether there should be a vote on the affiliation during the meeting was unanimously affirmed. After a short discussion the majority of participants (>30), with 4 people abstaining, voted for NTM-NET becoming a branch of the TB-NET.

The following session on Case Control Study on Predisposing host and environmental factors for NTM-infections was chaired by **Alexandra Nieters** and **Esther van de Vosse**.

- **A.Nieters** introduced the idea to start a Multinational epidemiological study of NTM infections among children and adults with a prospective follow-up of patients and recruitment of controls. She presented biological rationale to perform pilot studies in two areas, epigenetics of candidate genes of anti-mycobial immune response and micronutrients with specific focus on Vitamin D. These pilot studies with European partners could then be used as starting point to apply for international funding for a larger multi-center molecular epidemiological study. She emphasized the benefit to start a European NTM collaboration with long-term goal of a NTM-biobank. The interest in Vitamin D was shared by several members of the audience.
- **Martin Schumacher** (Freiburg, GER) gave an overview of Statistical Modelling in rare diseases and presented sample size requirements for the exploration of the study questions with sufficient statistical power. He illustrated why international collaboration is urgently needed in the rare disease context of NTM infections and suggested affiliation with an existing network as an option to share resources and include more patients.
- Annette Pohl (Freiburg, GER) then talked about Ethical issues in multicentre studies. She introduced some of the regulations and laws that govern clinical studies internationally. She also pointed out that the legal situation is very diverse in Europe and even between different parts in Germany. She spent the last part of her talk on some information about ethical issues pertaining to biobanking.

During the final discussion several research projects and ideas were discussed and putative participants and/or principal investigators were identified. It was decided to open a password protected NTM-workbench using the Wiki-format on the CCI website to further discuss and build up these research projects and ideas, to ease further communication between the group and to propose new projects or ideas to the NTM-group. Research projects of the NTM-NET that have reached proposal status will be proposed to the steering committee of the TB-NET by Dirk Wagner, who is the SC-member of the TB-NET. Dirk Wagner concluded the meeting with farewell remarks.