BDIAP Fellowship report by Janina Luisa (JL) Wolf

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As an external research rotation at the Leiden University Medical Center (LUMC) in the Netherlands I did my research internship for a half year at the Erasmus University Medical Center (EMC). For one of my research projects at the EMC I applied for the BDIAP Educational Fellowship.

General background of my topic:

Congenital pulmonary airway malformations (CPAM) belong to the most often diagnosed congenital lung malformations (up to 40%) with an incidence of approximately 1:30000 live birth. It is a benign hamartomatous lesion based on abnormal airway branching during lung morphogenesis resulting in the appearance of lung cysts. Nowadays CPAM are often identified during routine prenatal ultrasonography. After ~~birth~~birth, the majority of patients remain asymptomatic, nevertheless about ¼ will present with symptoms as breathing difficulties and recurrent infections. CPAM are classified by Stocker, according to level of the branching duct with type 1 and type 2 CPAM being the most common. Within these lesions in approximately 1/3 of (predominantly type I) are one or multiple areas of mucinous proliferations present. These lesions are thought to be a precursor of mucinous adenocarcinoma in situ or even mucinous adenocarcinoma. In these proliferations oncogenic KRAS mutations have been described. Within this subgroup a small percentage of these patients have developed mucinous adenocarcinoma out of these mucinous proliferations.

The topic was not chosen by myself. I got the offer from my supervisor Dr. Jan von der Thüsen. I hadn't heard of CPAM before, not even during my pathology internship, so I became interested these lesions and the fact that mucinous adenocarcinomas can develop out of them and be already present in children and/or young adults. This topic also fit in with my other project of pulmonary adenocarcinomas for which I came to the Erasmus MC.

The aim of the study was to investigate the potential of various immunohistochemical and genomic biomarkers to predict the presence of mucinous proliferations in CPAM. Therefore, archival CPAM tissue samples were re-assessed and underwent immunohistochemical analysis using a panel of differentiating markers (TTF1/CDX2/CC10/MUC2/MUC5AC/p16/p53/DICER1). In each sample, intensity of immunohistochemical staining was assessed separately in normal lung tissue, CPAM and mucinous proliferation (MP) tissue, using a semi quantitative approach (H-score). Likewise, next-generation targeted sequencing of known adult lung driver mutations, including KRAS/BRAF/EGFR/ERBB2, was performed in all samples with MP and in control samples of CPAM tissue without MP as well as normal lung tissue. 25 samples of CPAM type 1 and 25 CPAM type 2 were analyzed. Mucinous proliferations were found in 11 samples. They were all characterized by strong MUC5AC expression and all carried a KRAS mutation in the MP and the adjacent non-mucinous CPAM tissue, while the surrounding normal lung tissue was negative. By contrast, in less than half (5 out of 12) control samples lacking MP, the CPAM tissue also carried a KRAS mutation. KRAS mutations in non-mucinous CPAM tissue may identify lesions with a potential for malignant degeneration and may guide histopathological assessment and patient follow-up.

My role in this project was to collect all available pathology data and build a database. Therefore, I performed a search strategy in our department which offered us a sizeable dataset of 50 cases in the past 30 years. Most studies on CPAM are small case studies or case reports with a review of the literature. So, a database with 50 cases was great to work

with, also in terms of statistical analysis. I worked together with a colleague from Clinical Surgery who added clinical and radiological information to the database and who performed the statistical analysis. I reviewed all available HEs of the selected cases together with my supervisor Dr. von der Thüsen. While reviewing the material we focused of the following parameters: do we agree with the diagnosis? do we agree with the type of CPAM? presence or absence of mucinous proliferations, presence or absence of inflammation, type of inflammation (chronic-active, chronic, presence of foamy macrophages and mucous) and lining cell-type of cyst. (tall columnar-ciliated cells, non-ciliated columnar cells, cuboidal cells, flattened alveolar cells).

In a second step on all selected cases immunohistochemical analysis was performed. This was done by our research laboratory. I wanted to learn cutting and staining slides, but due to lack of time (I was just for 6 months at the EMC) it was fortunate that the lab did the work for me. By using the H-score I evaluated all immunohistochemically stains. If there was difficulty with interpretation, I also consulted my supervisor. In parallel I selected a subset of CPAM cases with and without mucinous proliferations for molecular analysis. I annotated all areas of interest on the HE and gave the stains again to the laboratory who did the analysis. The interpretation of the findings was done together with my supervisor. For the paper I wrote the material and method part and made a concept of the results and interpretation part. The introduction and statistical analysis were done by my clinical colleague. On the data presentation and interpretation, we worked together. During the writing process we were always in close contact. I really enjoyed working together with the colleague from the paediatric surgery, getting some clinical input as well as a clinical point of view on the project. But I also appreciated the close cooperation with my supervisor Dr. von der Thüsen and the fact I could easily walk into his room for consultation.

The immunohistochemical results I got, weren't that surprising in general. The mucinous cells stained as early described in case reports positive for MUC5ac and CDX2 and negative for TTF1 and MUC2. No aberrant staining in p53, DICER1 and p16 were found, as expected. Actually, I was a little bit disappointed by the marker CC10. It is a club-cell marker, and I had much hope in this marker to help me to differentiate between the different CPAM types as they develop at a different level of the bronchial tree. But no correlation was seen between type of epithelium, presence of CC10 and the type of CPAM. It had otherwise become a very nice marker in objectivizing the CPAM type. CC10 has not earlier been used as a marker for classifying CPAM. So, there was no experience yet with this marker. We (Jan and I) hypothesized, that following the branching hypothesis, hoe deeper the branching, the more club cells should be present. (Less club cells in < CPAM type I, < type II, < type III, <type IV < more club cells) But this hypothesis didn't fit the reality. In both, CPAM type I and II we experienced a heterogenic expression of club cells which were difficult to interpret. Reasons for this might be that we only had type I and II CPAM in our dataset, that the dataset was still too small and not sufficiently varied, that the marker isn't sensitive enough to discriminate between type I and II CPAM or that there isn't a gradient at all, because of disrupted branching physiology.

But the molecular findings were great, not that we found a KRAS mutation in the mucinous cell proliferation (this had already been described earlier) but that we found the same KRAS mutation in the non-mucinous CPAM tissue! And this is a new and surprising finding which led us to the hypothesis that the presence of a KRAS mutation in CPAM tissue might be suggestive for the presence of mucinous proliferations somewhere in the CPAM, even if we missed it due to a sampling error. We couldn't prove the hypothesis by embedding and cutting everything because the residual material of the macroscopic specimens had already bene disposed of, but the fact that the same mutation was present in the CPAM and the mucinous proliferations (isolated from two different slides to prevent contamination) was quite suggestive. There is some controversy how to handle CPAMs. Some countries prefer to remove them because of the potential of malignant degeneration, other countries on the other hand, such as the Netherlands, follow a more conservative mindset. It is not known yet if all CPAMs with a KRAS mutation will show malignant degeneration in the future, and therefore need to be removed, but we have one case in our database concerning a 30-year-old woman who presented with a CPAM type 1 and within the CPAM an invasive mucinous adenocarcinoma, harboring the same KRAS mutation as the CPAM tissue. Screening for the presence of KRAS mutations in small tissue biopsies in children presenting with asymptomatic CPAM may be of additional help in guiding the surgeons to remove these lesions or do a close follow up of these patients.

The majority of the project was based on histomorphology and immunohistochemical findings correlated with genetics and clinical information.

The research project was a great experience to me. It demonstrated on different levels that our work as a trainee/young pathologist isn't black and white. There are a lot of grey zones passing our microscope, which we have to accept and to interpret in the right context. For example, discussions about the type and classification of CPAMs. Even if we follow the classification system, looking at the size of the cyst and the morphology, it wasn't always that clear and easy to find a consensus, once my supervisor had to convince me that it is a CPAM and vice versa. The same applies to the assessment and the interpretation of the immunohistochemical markers, with a focus on the CC10. How many positive cells do we need to categorize the lesion? How to interpret heterogeneity in staining pattern and how to handle specimens with a cell morphology and cyst size which doesn't fit to the CC10 staining? It is sometimes unsatisfactory that a in theory potentially good idea doesn't work, but on the other hand, it challenges us to think forward and look for another solution. Pathology isn't black and white, and we can't make it black and white performing some research, but I can set some steps to interpret and classify lesions better, and this makes research a great thing to me.

On my work itself, COVID had not such a large impact. As a trainee and now as a young pathologist, I had to be present in the hospital and was able to continue with my research. For me, COVID had more impact on the meeting/congress. Everybody seemed so far away. I really enjoyed meeting the BDIAP community live including all the other fellowship members, to exchange the experiences, unfortunately this wasn't possible.
What concerns the presentation, I also had to get used to recording it. Everything was new, and I prefer more personal contact. But to be honest, there was at least one advantage with the pre-recording. I could redo it as often as I felt the need for it, not to blame all the people who worked directly and indirectly on this project. But at the same time, this was also the biggest disadvantage: it took me 23 hours to get it fixed and sent to the ESP. But afterwards, it saved me a lot of fuss, and could enjoy the online congress. But to be honest, I hope that there will be some time again to meet live, say "hello" and thank you personally for supporting my research!