Renal and Pulmonary Aspects of Birt-Hogg-Dubé syndrome

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Chapter 4.1.

Summary of the thesis

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Before the introduction of DNA-based diagnosis in the 1990's, hereditary tumour syndromes could only be recognized on the basis of clinical characteristics. These characteristics included typical features in the index patient and a positive family history. Since the 1990's the genetic background of most hereditary tumour syndromes has been identified. This allowed diagnosing these conditions based on germline mutation analysis and presymptomatic DNA testing in healthy at-risk family members. Moreover, diagnostic testing for these syndromes could now be performed in clinically equivocal cases, for example, in cases with only one or two minor features of the condition. Indeed, DNA testing showed that the hereditary syndromes, previously defined by the occurrence of multiple syndromic features had a much wider and more variable clinical spectrum than originally recognized. Finally, insight into the molecular pathogenesis in the syndromes has led to the development of targeted therapies aimed at the deranged signaling pathway or pathways involved. These developments have even led to the renaming of hereditary tumour syndromes from a clinically based definition to a DNA based definition, for example *PTEN* hamartoma tumor syndromes has replaced the former name of Cowden disease.

Birt-Hogg-Dubé syndrome is a good example of the developments outlined above. In 1977, the dermatologist Birt described an extensive kindred, investigated in cooperation with his colleagues Hogg, pathologist and Dubé, internist. The affected family members showed typical benign skin pathology, described by the authors as fibrofolliculomas, trichodiscomas and acrochordons. The pedigree showed an autosomal dominant inheritance pattern with high penetrance, in which most affected individuals developed skin lesions from the age of 25 years onward. Subsequently, it was recognized that renal cell cancer and spontaneous pneumothorax (SP) were part of the clinical syndrome. In 2002 the associated folliculin (*FLCN*) gene was identified. DNA testing now allowed confirmation of the diagnosis at the DNA level and DNA testing could be diagnostic in cases in which BHD was suspected. For *FLCN* mutation carriers surveillance for renal cancer was recommended aimed at the early detection and treatment of BHD associated renal cell cancer.

Although the fibrofolliculomas seem to be specific for BHD, both SP and renal cell cancer generally occur as a sporadic disease. Indeed, the clinical features of SP in BHD cannot be easily distinguished from those occuring in the common sporadic primary form. Renal cell cancer in BHD may show the typical characteristics of hereditary predisposition, i.e. early age at diagnosis, bilateral and multifocal disease and mixed histology patterns, but also common unilateral and unifocal clear cell renal cell

cancer in a middle-aged or elderly patient may be due to BHD. DNA testing has been performed in apparently sporadic RCC cases enriched for early onset of disease and familial occurrence and indeed *FLCN* mutations were found in apparently non-syndromic cases. In several studies it has been demonstrated that familial occurrence of SP without evidence of skin or renal manifestations can be due to germline *FLCN* defects. Thus, the skin features that originally defined BHD may be absent in *FLCN* mutation carriers who present with either renal or pulmonary manifestations or are healthy family members of an index case. Here we summarize and discuss the most important renal and pulmonary results of this thesis.

Part one: Pulmonary manifestations.

In **chapter 1.1.** we hypothesized that the development and natural course of lung cysts in patients with BHD is still unclear and the relationship between the cysts and development of SP has not been fully clarified. Based on the follow up results of thoracic imaging in 6 patients with Birt-Hogg-Dubé syndrome, we state that the pulmonary abnormalities of BHD patients are not due to a progressive degenerative disease. The decreased potential for stretching of the cysts' wall and the extensive contact with the visceral pleura are likely to be responsible for rupture of the cyst wall resulting in the increased risk for SP in BHD patients. Therefore we evaluated the reproducibility of measurements of size and number of pulmonary cysts on CT in 6 patients with BHD on baseline thoracic CT and in follow up CT. The main finding of this study was that within a period of 44 months there was no increase in size or number of pulmonary cysts. If cyst formation and SP are signs of a degenerative disease in BHD patients one might expect to see a higher prevalence of SP in older patients with BHD, which has not been reported in literature.[1] This differs from other diseases with cystic changes such as pulmonary lymphangioleiomyomatosis (PLAM) and pulmonary Langerhans cell histiocytosis (PLCH). Both are known to be progressive disorders.[2] Based on the observations in this manuscript we suggested that it is unlikely that the pulmonary abnormalities of BHD patients are due to a progressive degenerative disease. It is much more likely that the trend of development and recurrence of SP in BHD is related to the lack of possibility of epithelial layers to stretch if forced to do so by connection to the visceral pleura.

Only a few studies in current available literature describe the thoracic CT appearance in BHD patients, and are usually limited to several sporadic case reports or small retrospective studies with a small number of included patients. What the relationship between lung cyst characteristics and the development of (recurrent) SP is, has not been described and therefore we evaluated the possible relationship between cyst characteristics and SP in BHD patients. We hypothesized that chest computed tomography (CT) in this patient group might therefore be an useful tool for choice of treatment when developing a SP and might also play a role in advice of lifestyle. Therefore we evaluated in **chapter 2.2.** the findings of chest CT in a group of BHD patients with a history of (recurrent) SP and compared these patients with a group of BHD patients without a history of SP. We evaluated the radiological results of in total 61 patients, the largest study so far.
We only found a significant difference ($p<0.001$) in number of cysts between the group of BHD patients with a history of SP, compared to patients without a relationship of SP. We found no relationship ($\rho=-0.027$) between age and the number of cysts, which confirms the findings of our study as summarized in **chapter 1.1**. Therefore we suggested that BHD patients with a history of SP lead to more possibilities to suffer from cyst rupture than the non-SP patients. As thoracic CT does not always show detectable lesions, which are visible during VATS, a comparable study between radiological findings and in vivo VATS might therefore be necessary.[3]

Based on the fact that over 90% of BHD patients have cysts in basal parts of the lung, we hypothesized in **chapter 1.3**. that the use of a low dose chest CT might be an effective way to detect this syndrome in patients presenting with an apparently isolated PSP. This is important as its inheritance is autosomal dominant, and is associated with a risk of renal cell cancer of approximately16-30%. Therefore we included 46 patients, 19 with a proven *FLCN* mutation and 27 without an identifiable *FLCN* mutation, with a history of spontaneous pneumothorax. We found a higher prevalence of recurrent episodes of SP among patients with a proven pathogenic *FLCN* mutation, and also a higher prevalence of episodes, number of cysts. On thoracic CT the distribution, location and size differed significantly from patients without BHD syndrome. As we found a majority of cysts with a size < 2cm, this might explain why these abnormalities cannot be detected using standard erect chest X-ray. It is likely that in addition to air trapping other mechanisms play a role in the development of a pneumothorax. In the apical parts of the lung the pleural stress is high and

abnormalities in that area are likely to increase the risk of rupture.[4] In BHD-patients the majority of cysts are located close to the pleura in the lower lobes, which makes it likely that the wall of cysts connected to the visceral pleura ruptures easily.[5] Important for this is the lack of stretching possibilities of the wall of lung cysts of BHD patients.[6] As such this study is the largest study so far in literature, the main limitation remains the small number of patients we included. As this syndrome is relatively rare, it is difficult to gather a large cohort of patients. Although there seems to be a clear distinction on thoracic CT between BHD patients and patients without BHD, the rarity of this syndrome may still lead to unawareness among doctors who have to evaluate these thoracic CT's. Despite lacking information in this study on all clinical information regarding smoking history, familial inheritance on pneumothorax and prior (surgical) treatment of pneumothorax , we suggested that the radiological distinction between BHD and patients without BHD can easily be made on a low dose CT scan of the thorax.

The first episode of PSP usually occurs in the third decade of life in males, who are often taller than age-matched controls, and the majority has a history of smoking. Smoking increases the risk of PSP more than 100 times.[7] PSP diagnosis is usually based on history and confirmed by a standard erect chest X-ray during inspiration. Although we show in **chapter 1.2.** and **chapter 1.3.** a significant difference in thoracic imaging among BHD patients and patients without BHD despite a history of SP, it is radiologically still difficult to distinguish between BHD and smoking as a cause for SP. This difficulty is discussed in **chapter 1.4**. Therefore we describe a possible role for TTF-1 staining of the inner cyst wall, which might be specific for BHD.

In **chapter 1.5.** we assessed the relationship in time between air travel or diving and the occurrence of spontaneous pneumothorax in a large cohort of BHD patients with a proven pathogenic *FLCN* mutation. A questionnaire was sent to a cohort of 190 patients and the medical files of these patients were evaluated. In total 158 (83.2%) patients returned the completed questionnaire. Sixty-one of 145 patients who had ever travelled by airplane had a history of SP (42.1%), with a mean of 2.48 episodes (range 1-10), 24 (35.8%) had a history of bilateral episodes. Thirteen patients developed SP <1 month after air travel and 2 patients developed a SP <1 month after diving. Symptoms possible related to SP were perceived in 30 patients (20.7%) after air travel, respectively in 10 patients (18.5%) after diving. Based on the results reported in this chapter, we suggest that exposure of BHD patients

to considerable changes in atmospheric pressure gives an increased risk of developing a symptomatic pneumothorax. Symptoms reported during or shortly after flying and diving might be related to the early phase of pneumothorax.

The literature regarding the risk for SP after diving in patients with lung cysts is extremely limited. The British Thoracic Society (BTS) guideline recommends diving to be permanently avoided after an episode of spontaneous pneumothorax unless the patient has undergone bilateral surgical pleurectomy and the lung function and postoperative thoracic CT are normal.[8] [9] [10] Our results are comparable to the study results of the interstitial lung disease lymphangioleiomyomatosis with an incidence of 1.1 pneumothoraces per 100 patients.[11] [12]

Based on the study of Ren et al, which showed in 9.8% of cases a pathogenic *FLCN* mutation, among 102 patients with apparently primary SP (PSP), we evaluated in **chapter 1.6.** the prevalence of BHD among patients with apparently primary spontaneous pneumothorax (PSP).[13] Among the 40 patients with apparently common PSP, three had pathogenic germline *FLCN* mutations and one of these had a positive family history for pneumothorax. All three patients had multiple basal lung cysts. Asymptomatic renal cell cancer was detected in a first- degree family member of an identified BHD patient. The main limitations of this study were the low response rate and a possible selection of cases.

The response rate was low, since only 40 out of a total group of 316 patients (9.9%) were fully examined. Patients who were invited for the study may preferentially have opted for this possibility due to certain characteristics for example young age at diagnosis, high recurrence rate or a positive family history for the disease. In addition, after being informed on the characteristics of BHD patients with skin lesions or with a personal or family history of pneumothorax of renal cancer may have encouraged individuals to participate. As the results of the group of 40 patients is in line with the much larger study by Ren[13] and colleagues in consecutive cases with PSP, selection bias may not have been a major factor in this study.

The recurrence rate of SP in BHD has been described in literature to be as high as 75%, therefore we evaluated the effect of different types of treatment in **chapter 1.7.** Current BTS and ACCP guidelines do not describe the treatment of SP in BHD patients as a separate entity. In this study we compared

the results of treatment in a comparable group of BHD and non-BHD patients with (recurrent) SP. We found a recurrence rate of 64.5% after conservative treatment and a recurrence rate of 11.1% after invasive treatment of SP in BHD patients. This recurrence rate was significant higher when compared to patients without BHD. Therefore invasive treatment seems to be the better option for BHD patients with (recurrent) SP. Our results suggest that SP in BHD is associated with a high recurrence rate after conservative treatment and an invasive therapy would therefore be the best approach in this group.

Part two: Renal manifestations.

In **chapter 2.1.** the clinical data of 115 *FLCN* mutation carriers from 35 BHD families are evaluated. Among 14 *FLCN* mutation carriers who developed renal cancer 7 were <50 years at onset and/or had multifocal/bilateral tumours. Five symptomatic patients developed metastatic disease. Two early-stage cases were diagnosed by surveillance. The majority of tumours showed characteristics of both eosinophilic variants of clear cell and chromophobe carcinoma. The estimated penetrance for renal cancer and pneumothorax was 16% (95% minimal confidence interval: 6-26%) and 29% (95% minimal confidence interval: 9-49%) at 70 years of age, respectively. The most frequent diagnosis in families without identified *FLCN* mutations was familial multiple discoid fibromas. Based on these results we confirm the importance of surveillance for renal cancer in BHD patients. As renal tumours in BHD patients do not evidently differ from sporadic tumours in growth or histological pattern, it remains difficult to distinguish between BHD and sporadic renal cancer.

The prevalence of BHD among patients with apparently sporadic RCC is unknown, as the histological subtype and clinical presentation in BHD are highly variable. In **chapter 2.2.** we show the results of our retrospective study among patients diagnosed with sporadic RCC, wherein we retrospectively scored for the presence of lung cysts on thoracic CT. We performed *FLCN* mutation analysis in 8 RCC patients with at least one lung cysts under the carina. No mutations were identified. We compared the radiological findings in the *FLCN* negative patients to those in 4 known BHD patients and found multiple basal lung cysts were present significantly more frequent in *FLCN* mutation carriers. We therefore advise that in all RCC patients at least a concise family history is taken for the presence of RCC or SP and the skin is evaluated for the presence of fibrofolliculomas. In the presence of a positive

family history (SP or RCC) or multiple basal lung cysts further investigation of BHD is indicated (e.g. by dermatological evaluation or by DNA testing). The difficulty in unmasking BHD patients in apparently sporadic RCC patients is illustrated in this study by the negative family history for pneumothorax and RCC in the two *FLCN* mutation carriers.

In current literature renal surveillance in BHD is recommended, but the optimal imaging method and screening interval remain to be defined. In **chapter 2.3.** we retrospectively evaluated the compliance to, and the outcomes of renal cancer surveillance in patients diagnosed with BHD in two centers. Screening data of 199 patients diagnosed with BHD in two hospitals were collected. All available renal imaging follow up data and the medical records of 23 BHD patients with renal cell carcinoma (RCC) were collected. Initial screening was performed in 171/199 patients (86%) and follow up data were available from 117/171 patients (68%). The total follow-up period was 499 patient years. Of the patients that performed follow-up screening, 85% was screened at least yearly and 96% at least every two years. A medical history of RCC was present in 23 patients, 38 tumours were diagnosed with a mean age of the diagnosis of the first tumour at 51 years. In 21 tumours ultrasound (US) was performed. Eleven tumours, sized 7-27 mm, were visible on MR or CT and not detected using US. This study indicated that compliance to renal screening is relatively high and that US might be a sensitive, cheap and widely available imaging modality for detecting clinically relevant renal tumours in BHD patients, since no tumours exceeding 3 cm were missed with US.

Part three: Relevant case reports and case series

In chapter 3.1. we evaluated a patient with BHD that developed pneumothorax following flying and was manifested later. We consider the pressure changes during the subsequent flights as potential trigger for initiating rupture of a subpleural cyst, this implies that the interval between air travel and the diagnosis is important. In other studies 50% of cysts were located in the subpleural area and less than 5% abut on bronchioles. So if a cyst would be connected to the bronchial tree, the size of connection to the airways is very small, resulting in small volumes of air transported into the pleural cavity. Therefore it will probably take a long time before troublesome symptoms are present. Also

spontaneous resolution of a small pneumothorax after rupture of a subpleural cyst may occur if there is no active transport of gas into the pleural cavity. Based on this theory we concluded that BHD patients who have minimal chest symptoms after the first flight should be checked for pneumothorax before the return flight.

Although clinical manifestation usually appears after the age of 20, we discussed in **chapter 3.2.** two cases of BHD wherein episodes of (recurrent) pneumothorax occurred from the age of 14. Lung cysts were seen in both patients, mainly in the basal parts of the lung. SP has been reported only twice in pediatric pathogenic *FLCN*-mutation carriers. [14] [15] As SP in the pediatric population is relatively rare, BHD should be considered as underlying cause, especially when there is a positive family history for pneumothorax. Easy accessible genetic testing for BHD in pediatric patients with (recurrent) spontaneous pneumothorax should be performed, even when skin manifestation is absent. Based on this case series, more research on the prevalence of BHD in the pediatric population with a history of (recurrent) spontaneous pneumothorax might be needed.

In **chapter 3.3** we describe the case of a former Olympic swimmer who was referred to our hospital due to recurrent episodes of pneumothorax. Although the patient never smoked and the patient had multiple recurrences of SP, BHD was not suggested in this patient. This case shows the difficulty of recognizing this syndrome and the difficulty to distinguish this syndrome from common PSP.

An illustrative kindred is presented in **chapter 3.4.** in which the index patient had recurrent episodes of pneumothorax without apparent skin lesions or renal abnormalities. He had bilateral basally located lung cysts. Family members had fibrofolliculomas, lung cysts, pneumothorax and clear cell renal cancer. The described family highlights the importance for doctors to ask for family history regarding pneumothorax and renal cancer.

In **chapter 3.5.** we evaluated a patient who presented herself at the dermatologist with skin fibrofolliculomas. Based on the fibrofolliculomas BHD was suspected and an abdominal CT was performed. The abdominal CT showed a solid intrapolar tumour in the lower pole of the right kidney.

We concluded in this chapter that BHD should be considered when facial fibrofolliculomas are diagnosed and consequently relatives should be stimulated to be screened for genetic testing.

A patient with apparently sporadic chromophobe renal cell cancer is discussed in **chapter 3.6.** As the patient had a positive history for spontaneous pneumothorax and renal cancer, the patient was suspected for BHD syndrome, which was confirmed after molecular testing. We evaluated in this chapter the importance of recognizing this autosomal dominant cancer disorder when a patient is presented at the urologist with a positive family history of chromophobe renal cell cancer, or a positive familial history for renal cell cancer and pneumothorax.

Finally, in **chapter 3.7** we describe a patient who was referred to the clinical geneticist due to fibrofolliculomas. Although thoracic CT showed no lung cysts, the patient had a history of recurrent episodes of pneumothorax. As BHD was suspected, an additional abdominal MRI was performed which showed an asymptomatic tumour in the left kidney. A *FLCN* mutation was found in this patient, but the pathogenic mutation was absent in both parents. This is the first publication that describes a de *novo FLCN* mutation. *De novo FLCN* mutations are probably rare, but might be under-diagnosed. We suggest that BHD should be considered in patients with a negative family history who present with one or more of the syndromic features.

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