Presentation and early detection of posttransplant lymphoproliferative disorder after solid organ transplantation
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Summary, Discussion & Future directions
Posttransplant lymphoproliferative disorder or disease (PTLD) encompasses a heterogeneous group of lymphoproliferative diseases after solid organ transplantation. It ranges from an Epstein-Barr virus (EBV) driven polyclonal proliferation resembling infectious mononucleosis, to a highly aggressive monomorphic proliferation indistinguishable from diffuse large B-cell lymphoma. PTLD is considered to be a complication of immunosuppression after transplantation, leading to decreased function of EBV specific T-cells, which, in turn, may lead to uncontrolled proliferation of EBV infected B-cells. Because PTLD often presents in a non-specific way in patients without symptoms, it is a major challenge to diagnose PTLD at an early stage. In this thesis, disease presentation, risk factors for PTLD development and methods for early detection are discussed. In addition, we investigated the value of fluorodeoxyglucose (FDG)-positron emission tomography (PET) for staging and response monitoring of PTLD.

In chapter 2, we analysed incidence, patient characteristics, clinical presentation and prognostic factors for treatment outcome and survival of PTLD in a cohort of adult patients transplanted at the University Medical Centre Groningen between January 1985 and December 2002. PTLD histology was reviewed and classified according to the modified WHO classification as proposed by Nalesnik in 2001. Out of 1354 kidney and 206 lung transplants, PTLD was diagnosed in 40 transplant recipients (2.6%), which was in concordance with incidences observed in other transplant centres. Lung transplant recipients had a significantly higher incidence than kidney transplant recipients (p < 0.0001, log rank test). In concordance with the literature, sites of disease presentation were highly heterogeneous, with the large majority (73%) presenting extranodally. Interestingly, early PTLD (within one year posttransplant) was clearly associated with allograft localisation. In lung transplant recipients, we showed that more than 50 percent of all PTLD during the first posttransplant year developed in the allograft, whereas allograft localisation was rarely observed after the first posttransplant year (<15 percent). Although not as evident as in lung transplant recipients, PTLD development in the allograft was also significantly more frequent in the first posttransplant year in kidney transplant recipients.

The pathophysiological mechanisms leading to this preferential allograft localisation of PTLD early after transplantation have not been resolved yet. One could hypothesise that these lymphomas are the result of EBV-infected donor B-lymphocytes transplanted with the graft, which subsequently escape host immune surveillance, ultimately leading to donor-derived PTLD. In concordance with this hypothesis, PTLD of donor origin tends to arise early after transplantation and is more often localised in or near the allograft without dissemination, as compared to recipient-derived PTLD. On the other hand, the majority of early PTLD after solid organ transplantation are of recipient origin, and allograft localisation of PTLD, even early after transplantation, is far from exclusively associated with donor origin. Therefore, an alternative hypothesis is required to explain why the majority of early PTLD is localised in or near the allograft. We hypothesise that continuing allogeneic stimulation of the host immune system by the allograft contributes to the development of PTLD, which is in concordance
with earlier ideas\textsuperscript{14}. The local inflammatory processes associated with this allogeneic reaction might lead to a promiscuous pro-inflammatory microenvironment, facilitating proliferation of latently EBV-infected autologous B-lymphocytes, ultimately leading to PTLD.

The same mechanism may be involved in PTLD developing in the allograft late after transplantation, in which chronic infection of the allograft could also contribute to this promiscuous microenvironment. An interesting observation in this respect is the strong relationship between EBV-infected proximal tubular cells of the transplanted kidney (chronic EBV nephritis), even months before the onset of PTLD, and subsequent localisation of PTLD in or near the graft\textsuperscript{15}. It has been suggested that chronic EBV infection of renal proximal tubular cells may participate in evoking a cellular immune response not only resulting in a damaged renal interstitium, but also leading to a local pro-inflammatory environment\textsuperscript{16}. This observation suggests that chronic EBV infection of renal proximal tubular cells, even months before the onset of PTLD, is not causally associated with the development of (recipient derived) PTLD, but acts as an inflammatory trigger, facilitating local pro-inflammatory processes, thereby facilitating PTLD development. It is unknown whether other viruses, such as the oncogenic BK virus (frequently observed in kidney transplant recipients\textsuperscript{17}) can also contribute to the development of PTLD as a result of creating a local inflammatory environment. It would be of interest to study this relationship in the near future.

Apart from allograft involvement, the most commonly affected extranodal site of PTLD is the gastrointestinal tract\textsuperscript{3,18}, as was the case in 27\% of our patients. There seems to be no relation between time of onset and development of PTLD in the gastrointestinal tract. However, given the high incidence of PTLD at this site, one could hypothesise, in analogy with the development of PTLD in the allograft, that continuous exposure to infectious agents triggers a local inflammatory response, ultimately leading to PTLD. An interesting observation -further supporting the hypothesis that PTLD is facilitated by a non-specific pro-inflammatory microenvironment- is the development of PTLD at sites of previous surgery\textsuperscript{19}.

In chapter 3a, we analysed the role of HLA mismatches between donor and recipient in the development of PTLD in a cohort of 1013 patients having received a first cadaver kidney transplant at our centre between January 1985 and December 2002. We found a highly significant association between PTLD development and mismatches at the HLA-B locus, but not at the HLA-A or HLA-DR locus. In a multivariable Cox regression model, hazard ratios increased from 1.4 (95\% CI: 0.5-4.1) with one HLA-B mismatch to 5.1 (95\% CI: 1.4-19.0) in case of two HLA-B mismatches. This association was independent of immunosuppressive induction, maintenance or rejection therapy, factors all strongly associated with PTLD risk.

We could not identify an association between specific HLA haplotypes and risk for PTLD development. However, numbers are probably too small to identify any relation between specific HLA-B mismatches or HLA haplotypes and risk for PTLD development.
We hypothesise that decreased surveillance by T-cells with dual specificity for EBV as well as for allo HLA-antigens on the allograft, can facilitate clonal expansion of B-cells latently infected with EBV (see figure 1, chapter 3a). Interestingly, HLA-B mismatches have also been identified as independent risk factor for the development of skin cancer after renal transplantation\(^2\), which suggests that the risk of HLA-B mismatches in the context of poor immune surveillance is not restricted to PTLD.

Because the number of patients was too small, we could not analyse the possible influence of HLA mismatches on PTLD development in lung transplant recipients. Moreover, results in lung and kidney transplant recipients may not be comparable because of differences in immunosuppressive regimens and the lack of pre-transplant matching in lung transplant recipients.

Our findings emphasise the need for larger (multicentre) cohort studies to investigate whether specific HLA-B mismatches and/or specific HLA haplotypes are associated with development of PTLD. Such studies might reveal specific HLA-loci or alleles involved in EBV presentation and give new insights in the pathogenesis of PTLD. If our observation is confirmed by others, closer monitoring of patients with HLA-B mismatches is justified.

In chapter 4, we investigated the value of FDG-PET for staging and treatment evaluation of PTLD. In contrast to other lymphoma types, in which FDG-PET has proven to be superior over conventional diagnostic methods for staging as well as treatment evaluation\(^2\)\(^{-}\)\(^2\)\(^\_\)\(^2\)\(^2\), only few data of FDG-PET in PTLD are available\(^2\)\(^3\)\(^;\)\(^2\)\(^4\). In 12 renal transplant recipients with a histologically proven diagnosis of PTLD, we found PTLD generally to be highly FDG avid. FDG-PET scanning proved superior compared with conventional CT scanning for staging as well as treatment evaluation. Additional sites of extranodal localisations of PTLD not visualized on CT scanning were observed in 50% of patients. In concordance with results of FDG-PET in other malignant lymphoma types\(^2\)\(^5\)\(^-\)\(^2\)\(^7\), FDG-PET scanning also had good predictive value for outcome after treatment. Our results indicate that FDG-PET may also be very useful for staging and treatment evaluation of PTLD. Finally, it might be helpful in patients suspected of PTLD (e.g. by rising EBV-DNA load). Obviously, in these cases histological confirmation will always be necessary given the fact that FDG-PET uptake is not restricted to lymphoid proliferations.

Given the small numbers, we could not correlate FDG-PET findings with specific PTLD subtypes. This might have been interesting because PTLD is a heterogeneous disease and increased standard uptake value (SUV) of PET positive lesions in non Hodgkin’s lymphoma correlates with aggressive subtypes\(^2\)\(^8\).

In chapter 5, we compared EBV-DNA load measurements in whole blood with measurements in plasma. The optimal specimen for measuring EBV-DNA in solid organ transplant recipients is not known\(^2\)\(^9\)\(^-\)\(^3\)\(^0\). A total of 100 consecutive plasma samples from 25 lung and heart-lung transplant recipients with detectable EBV-DNA load (> 2,000 copies/ml) in whole blood were analysed. Of all EBV-DNA positive whole blood samples, only 17 samples (18%) were also positive in plasma (here defined as >1,000 copies/ml).
In this chapter we concluded that whole blood, by virtue of its sensitivity, rather than plasma may be the preferable specimen for the detection of EBV-DNA in lung transplant recipients. This seems logical, as there may be an underestimation of EBV-DNA load in plasma. Whole blood contains all EBV-DNA (i.e. cell free and cell associated), whereas plasma contains only cell free EBV-DNA. Nevertheless, results have to be evaluated in view of a selection bias; only samples that were scored positive for the presence of EBV-DNA in whole blood were included. Hypothetically, samples negative in whole blood could be positive in plasma. However, given the results of our study, this seems very unlikely as 95% of all positive plasma samples had lower EBV-DNA loads than measured in whole blood.

One could hypothesise that EBV-DNA measurements performed in plasma are more specific than measurements in whole blood for the detection of already established PTLD. This hypothesis is based on the finding that EBV-DNA in plasma mainly consists of small (less than 181 base pairs) DNA fragments, that are considered to be derived from apoptotic B-cells as a result of cell lysis, which may be the case in PTLD. This is further supported by data from Wagner et al., who reported a sensitivity and specificity of 100 percent of positive plasma EBV-DNA measurements for the presence of PTLD.

In contrast to measurements in plasma, EBV-DNA measurements in whole blood are also influenced by proliferating B-cells containing EBV-DNA in the absence of PTLD and, consequently, probably without release of viral DNA. This might explain the observed low specificity of whole blood measurements for the development of PTLD. In this respect it is very interesting that also EBV-negative PTLD cases can develop simultaneously with a sharp increase in peripheral blood EBV-DNA load. These cases suggest that, although increased EBV-DNA load is generally considered to represent an increase in circulating EBV-positive tumour cells, high EBV-DNA loads might also represent a separate population of proliferating B-cells that may have nothing to do with possible development of PTLD. Instead, these proliferating B-cells may only reflect a general state of decreased T-cell surveillance in the transplant recipient.

The aim of EBV-DNA measurements in transplant recipients is to identify the patient at risk for PTLD development at an early stage. Pre-emptive interventions could then be applied. For this purpose, EBV-DNA measurements in whole blood seem to be more useful, as early EBV driven B-cell proliferation without already established disease can then be detected. Possibly, plasma EBV-DNA measurements might then be helpful in further differentiating between PTLD development and indolent B-cell proliferation. To further explore this hypothesis, it would be necessary to study serial EBV-DNA load measurements in both plasma and whole blood in a group of patients without pre-emptive treatment. However, questions can be raised on the ethical applicability of such a protocol in view of the already known risk for development of PTLD in patients with rising EBV-DNA load and the emerging data supporting the feasibility and positive effects of pre-emptive treatment (i.e. reduction of immunosuppression or infusion of EBV specific cytotoxic T-lymphocytes) on PTLD incidence.

In chapter 6, we studied the feasibility of whole blood EBV-DNA load guided reduction of immunosuppression as pre-emptive treatment for prevention of PTLD in
75 lung transplant recipients at different stages in their clinical course. It has already been reported that reduction of immunosuppression guided by EBV-DNA load might lead to a lower incidence of PTLD in paediatric liver transplant recipients\textsuperscript{35,36}. However, the use of such an approach has not been reported in lung transplant recipients so far, probably because of the severe consequences of possible rejection. Patients in this study were transplanted between 1990 and 2001 and followed for this study from June 1, 2001 until January 1, 2006. In case of an EBV reactivation, defined as two consecutive whole-blood EBV-DNA load measurements with a rising tendency, the last one exceeding 10,000 copies/ml, immunosuppression was tapered. Median time after transplantation was 4.25 years (range 0-10 years). During the study period, no primary EBV infections were observed.

The most important finding of this study was the fact that none of the patients with an EBV reactivation developed acute rejection following reduction of immunosuppression. Also, EBV reactivation (followed by subsequent reduction of immunosuppression) was not associated with acceleration of bronchiolitis obliterans syndrome (BOS) or worse survival in our patients. It seems, therefore, that reduction of immunosuppression is safe in case of EBV reactivation following lung transplantation.

During the observation period, one patient (1.5\%) developed EBV negative PTLD following an EBV reactivation despite reduction of immunosuppression. However, because of the heterogeneous composition of our cohort and the lack of a comparable control group, we cannot conclude that reduction of immunosuppression leads to a lower incidence of PTLD. Nevertheless, the observed incidence of PTLD (1.5\%) during the study period in our group is lower than that reported in the literature, also taken in account the interval between transplantation and the start of the study\textsuperscript{38}. It seems, therefore, that this approach is very promising for the prevention of PTLD developing late after lung transplantation.

**Future directions**

Early detection and possible prevention of PTLD will be a major challenge for the near future. In addition, a better understanding of the exact pathophysiologic mechanisms in PTLD development is warranted in order to improve or develop specific new therapeutic strategies. EBV-DNA load measurements, especially if serially applied in the individual patient, are important for the early identification of patients at risk for PTLD development. However, given the limitations of EBV-DNA load monitoring as predictor for PTLD, this can never be the sole parameter to rely on. Preliminary results with concomitant measurements of CTL responses are promising\textsuperscript{39,40}. Possibly, these combined methods might be helpful in better identifying the patient at risk for PTLD development and, subsequently, guide pre-emptive treatment. Prospective studies are needed to better define the exact value of these methods.
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