Identification of IG-clonality status as a pre-treatment predictor for mortality in patients with immunodeficiency-associated Epstein-Barr virus-related lymphoproliferative disorders

Immunodeficiency-associated Epstein-Barr (EBV)-related lymphoproliferative disorders (EBV-LPD), including the post-transplant lymphoproliferative disorders (PTLD), are aggressive hematologic malignancies which, despite improvements in therapy, including the use of anti-CD20 monoclonal antibody, result in considerable morbidity and mortality.[1-3] Retrospective analyses have revealed several clinical risk factors, including therapeutic interventions, that predict outcome in patients with EBV-LPD. However, pre-treatment risk stratification that can be used to guide therapeutic decisions remains difficult and algorithms are lacking.[1] Although morphology is regarded to be a cornerstone in therapy decision making, immunoglobulin (IG) clonality might help prognostication. Despite efforts to standardize the pathological classification of EBV-LPD,[4] neither histology nor IG clonality has been shown to consistently predict outcome.[5-7] Nevertheless, comprehensive IG clonality testing, with a high detection rate of clonality, allows an objective pathological parameter to be re-evaluated in risk stratification of EBV-LPD.[8]

We performed a retrospective analysis in a large multicenter cohort of 86 patients with EBV-LPD: 62 patients with an EBV-positive PTLD and 24 with another iatrogenic immunodeficiency related EBV-LPD. Patients from 2000-2012 were included in the analysis; patients' characteristics are presented in *Online Supplementary Table S1*. IG-gene clonality testing was performed by assessment of the IGH-V(D)J, -DJ as well as IGK (VJ and KDE) rearrangements using the BIOMED2 approach. This IG clonality assay has an unprecedented high detection rate[8,9] due to the complementarity of the PCR- targets with a sensitivity of each individual PCR of 5%-10% and the specificity of the IG-clonality assay of 94%.[10] In this study cohort, 20% of the clonal cases had clonal IGK and/or incomplete IGH-DJ rearrangements without having clonal IGH V(D)J-FR1, 2 or 3 rearrangements. These cases were found in both the EBV-positive PTLD and the iatrogenic immunodeficiency-related EBV-LPD group, and would have gone unnoticed when clonality testing was based only on assessment of the complete IGH rearrangement. Interpretation of the clonality findings was performed according to the EuroClonality guidelines.[11] Patients were classified as having either monoclonal or oligo/polyclonal EBV-LPD. Histology was examined by 2 experienced hematopathologists and designated either monomorphous or reactive/polymorphous (no Burkitt-or Hodgkin-type lesion was included) according to the WHO criteria.[4] In the majority of the cases, IG clonality was detected in multiple PCRs. There was no clear difference in clonality pattern in monomorphic subtype PTLDs *versus* the reactive/polymorphic PTLDs, albeit the last group tended to have more cases showing monoclonality with a polyclonal background, although this is not exclusively seen in this group. A statistical analysis was performed to identify pre-treatment risk factors for poor outcome defined as EBV-LPD-related mortality, with an emphasis on pathological features, but also clinical stage according to Ann Arbor, extra-nodal disease, age and underlying diagnosis.

Table 1. Univariable and multivariable analysis of risk factors for EBV-LPD related mortality.

Risk factor	EBV-LPD mortality	OR univariable (95%CI)	*P* univariable	OR multivariable (95%CI)	*P* multivariable
Age					
≥ 50 years	13/36 (36%)	2.6 (1.0-6.9)	0.08	3.6 (1.2-11)[2]	0.03
< 50 years	9/50 (18%)				
Sex					
Male	17/55 (31%)	2.3 (0.8-7.1)	0.2	–	–
Female	5/31 (16%)				
Diagnosis					
PTLD	20/62 (32%)	5.2 (1.1-24.5)	0.03	1.5 (0.3-8.7)[1]	0.6
Iatrogenic EBV-LPD	2/24 (8%)				
Stage					
II-IV	21/59 (36%)	14.4 (1.8-113.5)	0.001	13.8 (1.6-117.2)[2]	0.02
I	1/27 (4%)				
Extranodal disease					
Yes	12/46 (26%)	1.1 (0.4-2.8)	1.0	–	–
No	10/40 (25%)				
Morphology					
Monomorphic	15/45 (33%)	2.5 (0.9-6.8)	0.14	–	–
Reactive/polymorphic	7/41 (17%)				
IG clonality[2]					
Monoclonal	21/66 (32%)	8.9 (1.1-70.7)	0.02	6.6 (0.8-59.1)[2]	0.09
Oligo/polyclonal	1/20 (5%)				
EBV load at diagnosis[3]					
≥ log 3	17/45 (38%)	1.5 (0.5-4.3)	0.5		
< log 3	3/12 (25%				

P < 0.05 was considered statistically significant. OR: odds ratio. [1]Model with four covariates. [2]Model with three covariates. [3]Data on EBV load only present in 57 patients.

Univariable analysis using the Fisher exact test revealed multiple risk factors for EBV-LPD-related mortality; $P<0.05$ was considered significant (Table 1). This included PTLD as underlying diagnosis, disease stage II-IV and IG monoclonality, but not age 50 years or over, EBV load at diagnosis, and monomorphic histology. Next we performed a multivariable logistic regression analysis with the four variables having at least a P value of 0.10 in the univariable analysis. In the first model, incorporating all four risk factors, underlying diagnosis was not associated with EBV-LPD-related mortality ($P=0.6$), but the other three, including age 50 years or over, showed an association with $P\leq0.1$. Analyzing these three risk factors simultaneously in a second model revealed that age and disease stage were significantly related to EBV-LPD-related mortality (Table 1), and that IG-clonality showed a trend ($P=0.09$). Nevertheless, the high odds ratios at least suggest that the risk factors might all have had an impact and that significance was not established in all three of them due to the small cohort size.

Because diseases with a high mortality rate require prognostic factors that preferably identify all those at risk for mortality, we next investigated which of the pathological factors had the highest sensitivity and negative-predictive value for EBV-LPD-related mortality. Sensitivity, specificity, negative predictive and positive predictive value for EBV-LPD-related mortality in the total cohort were 68%, 53%, 83%, 33% for monomorphic morphology and 95%, 30%, 95%, 32% for monoclonal IG-gene status, respectively. When looking at PTLD cases separately, similar results were seen with the sensitivity and negative predictive value of monoclonal IG-gene status exceeding that of monomorphic morphology (95% and 89% *vs.* 70% and 74%, respectively).

The higher negative predictive value at least suggests that IG-clonality testing performs better than histological examination when used to identify patients that are not at high risk of death. So, patients can be identified that might not require prompt treatment, that is, those with oligo/polyclonal disease. Clonality testing, however, has a similar, but equally low, positive predictive value as histology. Therefore, establishing either monoclonality or monomorphic disease does not necessarily mean that a patient is at high risk for death from EBV-LPD, and therapeutic decision making based on clonality status alone might result in overtreatment.

Realizing the limitations of the multivariable analysis, and the very different clinical context of patient groups defined as either PTLD or another iatrogenic immunodeficiency EBV-LPD, the decision was made to analyze the two groups separately (*Online Supplementary Table S1*); this precluded comprehensive statistical analysis and, therefore, only descriptive statistics were used.

The subgroup of patients with PTLD consisted of 41 hematopoietic stem cell and 21 solid organ transplant recipients. The distribution of morphological subtypes was similar in the SOT and SCT subgroups with approximately 60%-65% monomorphic disease. In those patients presenting with monomorphic PTLD, 90% (35 of 39) had been classified as monoclonal EBV-LPD. The 4 monomorphic PTLD cases without monoclonal IG status all had oligoclonal EBV-LPD and displayed similar clinical features to the monoclonal cases. Mortality was high at 36% (14 of 39) despite the use of R/R-chemo (Figure 1A) More specifically, of the 14 patients who died, 13 had monoclonal and one oligoclonal EBV-LPD.

In patients with reactive/polymorphic PTLD, the IG-clonality status seemed to be of importance. Monoclonality resulted in an unfavorable outcome with a mortality rate of 33%, which is similar to that seen in the patients with monomorphic PTLD. However, a considerable number of deaths were caused by insufficient treatment. Five patients who died had not received R/R-chemo, probably as a result of inadequate risk assessment based on morphology, age and stage. In contrast, polyclonal reactive/polymorphic PTLD patients had a good outcome with modification of immunosuppression only (Figure 1A).

There were 24 patients with another iatrogenic immunodeficiency EBV-LPD, which involved 22 patients treated for inflammatory bowel disease, e.g. Crohn disease and ulcerative colitis. Extranodal disease involving the diseased colon itself was very common, 72% (16 of 22). Overall, EBV-LPD mortality was 8% (2 of 24). Ann Arbor staging seemed most predictive for outcome (Figure 1B). Stage I disease (n=16) was effectively cured by only modifying immunosuppressive therapy, sometimes complemented by surgical resection; the IG clonality status (44% monoclonal)

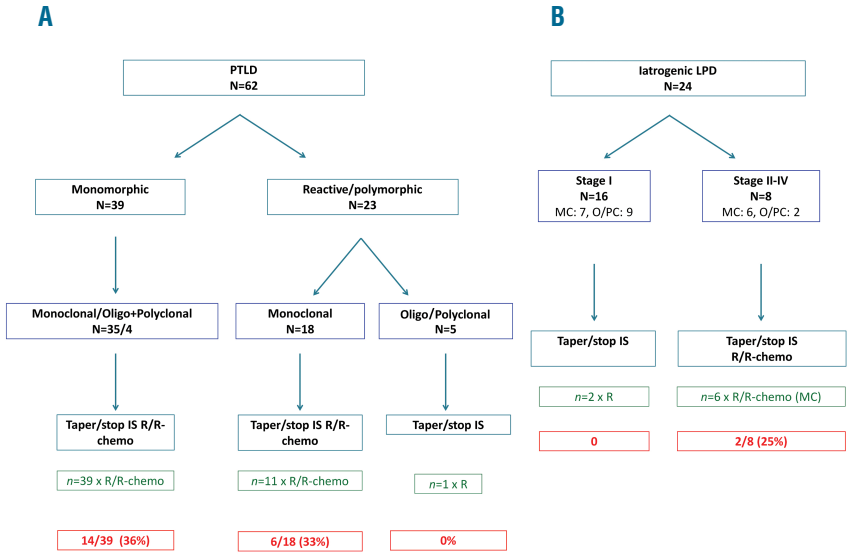

Figure 1. Flowcharts of the clinico-pathological features and patient outcome of patients with EBV-related lymphoproliferative disorders and separated by diagnose subgroups iatrogenic EBV-LPD and PTLD. The therapy that had been applied mostly is shown in the dark blue boxes. The number of patients who actually did receive rituximab alone or in combination with chemotherapy (R/R-chemo) are shown in the green boxes. Outcome is expressed as mortality rate and indicated in the red boxes. IS: immunosuppressant; MC: monoclonal; O/PC: oligo/polyclonal.

was not relevant. More advanced stages, II-IV (n=8), which proved monoclonal in 75% of the cases, required treatment with rituximab (R) alone or combined with chemotherapy (R-chemo), but there was still a mortality rate of 25% (2 of 8) (Figure 1B). The 2 patients who had died both had monoclonal disease and succumbed despite use of R/R-chemo.

Our analysis shows that IG-clonality status might be useful in the risk stratification and therapeutic decision making in patients with EBV-LPD in the setting of PTLD. Monoclonal, monomorphic EBV-LPD in PTLD requires early aggressive intervention; polyclonal reactive EBV-LPD may be managed conservatively. In IBD patients with EBV-LPD, low disease stage is more predictive of survival, regardless of whether or not the disease is monoclonal. The fast and full recovery of immunity with reduction of immunosuppressants expected in these patients, who have no additional immunological deficits, seems sufficient to achieve a remission. This contrasts with the situation of PTLD after transplantation where more profound and prolonged immune deficits arise from pre-treatment and conditioning therapy, which precludes control of EBV-LPD by a functional immune system on cessation of immunosuppressants.[12,13] Reactive/polymorphic and polyclonal PTLD probably reflects an earlier phase of the disease where there might be more time for immune recovery to occur, and so, even in the setting of transplantation, additional therapy can be reserved for those failing modification of immunosuppressive therapy.

Our analysis has several limitations that are related to the retrospective nature of the study and the limited sample size. Nevertheless, our findings appeal for future multicenter prospective studies that incorporate IG-gene clonality testing in a risk stratified approach to PTLD.

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