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Immunoglobulin abnormalities in 1677 solid organ transplant recipients. Implications for posttransplantation follow-up.

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A B S T R A C T

Background: Posttransplant lymphoproliferative disorder (PTLD) is a severe complication of solid organ transplantation (SOT). However, there is no consensus on PTLD screening methods. Gammopathies (GP), which occur in 10–25% of SOT recipients, have been linked to subsequent development of PTLD. Therefore, GP detection methods, such as serum protein electrophoresis (SPE), serum protein immunofixation (SIFE), urine protein immunofixation (UIFE) and the quantitative measurement of serum free light chains (SFLC) are candidate methods for PTLD screening.
Objective: We aimed to assess the frequency of PTLD and GP, association of GP with subsequent PTLD, allograft loss or death and the diagnostic performance of SPE/SIFE in PTLD screening. The main objective was to explore, whether GP detection methods can be used to enhance the efficiency of PTLD screening and to formulate a concise algorithm for posttransplantation (post-Tx) follow-up.
Methods: We performed a cohort study on 1677 SOT recipients with SPE/SIFE data who underwent kidney, liver, heart, pancreas, Langerhans islets or multiple organ transplantation at the Institute of Clinical and Experimental Medicine between 1966 and 2015. The median (IQR) of follow-up time was 8.0 (4.0–12.0) years.
Results: The frequencies of PTLD and GP in SOT recipients were 2.8% and 6.4%, respectively. The frequencies of transient GP, GP of undetermined significance and malignant GP were 33%, 63% and 4% respectively. The median time between SOT and GP detection was 2.0 (interquartile range 1.0–7.0) years. GP was associated with a significantly higher risk of PTLD, allograft loss and death, with hazard ratios (95% confidence intervals) of a 6.06 (2.51–14.64), 2.61 (1.49–4.6) and 1.99 (1.2–3.3), respectively. Additionally, GP was associated with 2.98-fold increased risk of allograft loss in kidney transplant patients. SPE diagnostic sensitivity and specificity for PTLD were 14.8% and 93.9%, respectively. PTLD was diagnosed more often and earlier if SPE/SIFE was included in the post-Tx follow-up.
Conclusions: GP after SOT is associated with a high risk of PTLD, allograft loss and poor survival. The combination of SPE, SIFE, SFLC and UIFE is optimal for GP detection. These methods aid in identifying patients who are at risk for PTLD or allograft damage and should be included in regular post-Tx follow-up.

1. Introduction

Posttransplant lymphoproliferative disorder (PTLD) is a severe complication of solid organ transplantation (SOT) with a mortality rate of 20–70% [1–4]. The cumulative incidence of PTLD ranges from 1% to 20% [1,3–6], depending on various risk factors, such as *Epstein Barr-virus* (EBV) *and Cytomegalovirus* (CMV) infection, age at transplantation (Tx), the type of organ transplanted, or the type and dosage of immunosuppressive medication [7–14].

PTLD is characterized by a heterogeneous clinical presentation, which may include late-stage organ damage but may also be limited to nonspecific symptoms (weight loss, lymphadenopathy, dyspepsia). The definitive diagnosis of PTLD relies on biopsy-based methods, which cannot be used for regular posttransplantation (post-Tx) screening. Therefore, there is an ongoing search for methods using peripheral blood samples, which possess low patient burden and offer sufficient diagnostic performance for reliable screening.

Gammopathies (GP), which occur in 10–25% of SOT recipients, have been linked to immunosuppressive treatments and the subsequent development of PTLD [5,6,15–23]. Thus, GP detection methods, serum

Abbreviations: CB/CC NHL, centroblastic/centrocytic non-Hodgkin lymphoma; CLL/SLL, chronic lymphocytic leukaemia/small lymphocytic lymphoma; CMV, *Cytomegalovirus*; DLBCL, diffuse large B-cell lymphoma; EBV, *Epstein Barr-virus*; GP, gammopathy; HTx, heart transplantation; IKEM, Institute for Clinical and Experimental Medicine, Prague, Czech republic; IQR, interquartile range; KTx, kidney transplantation; LTx, liver transplantation; MGUS, monoclonal gammopathy of undetermined significance; NHL, non-Hodgkin lymphoma; PTLD, post-transplant lymphoproliferative disorder; SFLC, quantitative measurement of serum free light chains; SIFE, serum protein immunofixation; SOT, solid organ transplantation; SPE, serum protein electrophoresis; Tx, transplantation; UIFE, urine protein immunofixation

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protein electrophoresis (SPE), serum protein immunofixation (SIFE) and quantitative measurement of serum free light chains (SFLC), have already been tested as candidate PTLD screening methods. However, previously published results have displayed heterogeneity, and no clear consensus on the implementation of these methods in PTLD diagnostics has been reached so far [5,6,15–20,24–35].

As a result, there is no comprehensive guideline for SPE, SIFE and SFLC use in SOT recipients. This favours ineffective ordering of tests and misinterpretation of results with incorrect management of GP patients.

2. Objective

We aimed to explore, whether GP detection methods can be used to enhance the efficiency of PTLD screening and to formulate a concise algorithm for post-Tx follow-up.

3. Data collection and patient characteristics

We performed a cohort study on SOT recipients with SPE/SIFE data who underwent Tx at the IKEM between 1966 and 2015. IKEM performs all major SOT types except for lung Tx. The laboratory results database was searched for SPE/SIFE results, and the patient history database was searched for sex, age at Tx, transplanted organ, date of Tx, PTLD type and date of diagnosis, allograft loss and patient death. The follow-up period was defined as the time from the first Tx to the last examination in 2015 or patient death. All patients provided informed consent to all diagnostic and therapeutic procedures used. The study was approved by the Ethics Committee of the IKEM under G14–08-26.

Three groups of SOT recipients were studied, referred to as cohorts A, B and C. Details on the patient characteristics are given in Table 1. The inclusion criteria are described below, patient recruitment flowchart is presented as Fig. 1.

3.1. Patient cohort A

First, a primary study cohort was enrolled. Only analyses performed by qualified technicians on a SEBIA Hydrasis 2 analyser with Sebia agarose gel SPE/SIFE kits, that were interpreted by 2 alternating medical doctors with experience in the field and with results given in a standardized format according to guidelines [36] were used. GP was defined as a distinct monoclonal, biclonal or oligoclonal band that was detectable by SPE, which was consequently confirmed and typed by SIFE. The development of GP over time was only assessed when > 1 SPE/SIFE examination was available. GP were classified as persistent when they were detected on at least two consecutive examinations and were present until the end of the follow-up. Otherwise, GP were classified as transient.

If lymphoproliferation was diagnosed, only cases that occurred > 6 months after Tx were enrolled as PTLD, to avoid misclassification between post-Tx and pre-Tx lymphoproliferation disease. Additional inclusion criterion was verification of PTLD by a pathologist, with WHO classification [37]. Also, 6 patients with lymphoproliferations not included in WHO PTLD types were enrolled (2 B-chronic lymphocytic leukaemia/small lymphocytic lymphoma, 1 mantle-cell lymphoma, 1 skin lymphoma, 1 centroblastic/centrocytic non-Hodgkin lymphoma, lymphoblastic diffuse lymphoma).

This resulted in 1677 SOT recipients who qualified, with pre-Tx and post-Tx data available in 480 and 1663 patients, respectively. This cohort was used for all frequency, time of first detection, GP clinical significance and diagnostic performance analyses.

3.2. Patient cohort B

Subsequently, using the IKEM Tx database, the exact number of patients eligible for screening, but without SPE/SIFE was identified, with a total of 2624 SOT recipients. Only patients who underwent Tx from 2005 to 2015 were enrolled due to standardized SPE/SIFE analytical methodology and interpretation in this period. This second cohort was enrolled to create a reference group without SPE/SIFE to perform post-Tx comparative analyses.

3.3. Patient cohort C

Finally, a subset of cohort A, which was comparable to the reference cohort B in regard to age, sex, year of Tx, length of follow-up and PTLD type was established, totalling 1028 patients. This enabled comparative analyses of PTLD diagnostics in patients with and without GP screening included in the post-Tx follow-up as well as the assessment of the current state of SPE/SIFE screening in SOT recipients.

4. Methods

4.1. Diagnostic performance of SPE/SIFE in PTLD patients

Method sensitivity, specificity, and positive and negative predictive values were calculated using SPE/SIFE results from a two-year period prior to PTLD diagnosis.

The usefulness of SPE/SIFE in PTLD diagnostics was assessed by comparing the number of PTLD cases diagnosed and time to PTLD diagnosis in two patient cohorts B and C.

4.2. Time-to-event analysis

To determine the relative risk of PTLD, mortality and allograft loss associated with the presence of post-Tx GP, we performed Cox proportional hazard regression and competing risk regression according to Fine and Gray [38].

Patients were included in the analysis from the date of Tx until the date of primary event (PTLD, death or allograft loss) or the end of follow-up (latest follow-up occurred November 16th 2016), whichever came first. In addition, we considered death as competing risk to diagnosis of PTLD or allograft loss using the Fine and Gray model. Time since entry to the study was the time scale for both regression models and we censored the patient contribution to the analysis at the date of last follow-up or death. To assess whether the effect of GP varies between Tx types, we performed subanalyses on patients with liver and kidney allografts. We could not assess other organs, due to small sample sizes in the GP group. Due to the time-lag between the index date and the diagnosis of post-Tx GP, the primary exposure was considered in both Cox and competing risk models as a time-varying covariate.

Seven regression analyses were performed. Three Cox analyses on the effect of GP on each primary event, two competing risk analyses on the effect of GP on PLTD and allograft loss, and four Cox subanalyses on the effect of GP on allograft loss or death – two in patients with kidney transplants and two in patients with liver transplants. All analyses were adjusted for age at Tx; adjustment for sex was regarded as unnecessary due to a statistically equivalent distribution of GP between men and women (chi-square for difference, $p = .483$) and non-significant estimates in the regression models. No variable violated the proportionality of the hazards assumption. Data were analysed using SAS version 9.4 (SAS institute, Cary, NC).

Results from the Cox and competing risk regression are presented as hazard ratios or subhazard ratios with 95% confidence intervals.

5. Results

5.1. Frequency and first detection of gammopathies

Frequency data are presented as the absolute counts and percentage of patients with SPE/SIFE findings. Longitudinal data are presented as the median and interquartile range (IQR).

Table 1
Patient characteristics.

Characteristics		Cohort A Primary (n = 1677)	Cohort B Reference (n = 2624)	Cohort C Subset of cohort A (n = 1028)
Year of Tx		1966–2015	2005–2015	2005–2015
Length of follow-up, median (IQR) of years		8.0 (4.0–12.0)	6.95 (4.3–10.3)	8.1 (5.3–10.6)
Sex	Male	1028 (61.3%)	1761 (67.1%)	652 (63.4%)
	Female	649 (38.7%)	863 (32.9%)	376 (36.6%)
Age group at transplant (years)	Median (IQR)	52.1 (41.4–59.4)	52.7 (41.4–60.9)	54.5 (44.9–61.3)
	< 18	28 (1.7%)	60 (2.3%)	12 (1.2%)
	18–50	689 (41.1%)	1070 (40.8%)	353 (34.3%)
	> 50	960 (57.2%)	1494 (56.9%)	663 (64.5%)
Tx organ	Heart	41 (2.4%)	447 (17%)	23 (2.2%)
	Kidney	723 (43.8%)	1678 (64.0%)	395 (38.4%)
	Liver	824 (48.5%)	253 (9.6%)	559 (54.5%)
	Liver + Kidney	24 (1.4%)	11 (0.4%)	21 (2.0%)
	Pancreas + Kidney	50 (3.0%)	235 (9.0%)	30 (2.9%)
	Other[a]	15 (0.9%)	Not included[c]	Not included[c]
PTLD cases	All	47 (2.8%)	17 (0.6%)	20 (1.9%)
	Early lesions	2 (0.1%)	1 (< 0.1%)	0
	Polymorphic PTLD	1 (0.1%)	1 (< 0.1%)	0
	Monomorphic B-cell	30 (1.8%)	11 (0.4%)	15 (1.5%)
	Monomorphic T/NK cell	4 (0.2%)	3 (0.1%)	3 (0.3%)
	Hodgkin lymphoma	2 (0.1%)	1 (< 0.1%)	0
	Other[b]	6 (0.4%)	0	1 (< 0.1%)
	Unspecified	2 (0.1%)	0	1 (< 0.1%)

[a] Pancreas, Heart + Kidney, Kidney + Langerhans islets, Liver + Langerhans islets, Langerhans islets.
[b] 2 B-SLL/CLL, CB/CC NHL, skin lymphoma, lymphoblastic diffuse lymphoma, mantle cell lymphoma.
[c] Analyses on patient cohorts B and C were performed only for 5 main Tx types.

Pathological SPE/SIFE findings were present pre- and post-Tx in 20 (4.2%) and 107 (6.4%) patients, respectively. Post-Tx monoclonal, biclonal and oligoclonal GP were detected in 71 (4.3%), 9 (0.5%) and 27 (1.6%) patients, respectively. GP was most common after heart transplantation (HTx), with 6 (15.4%) cases. The median time between Tx and GP was 2.0 (IQR 1.0–7.0) years. Details are given in Fig. 2 and Table 2.

The most frequent type of post-Tx GP was monoclonal, isotype IgG-kappa or lambda, both of which were detected in 24 (22%) GP cases (Fig. 3). The distribution was dependent on the type of Tx. Predominantly oligoclonal and monoclonal GP of the IgG lambda isotype were detected in LTx patients. The GP of kidney transplant (KTx) patients were mostly monoclonal of the IgG kappa isotype.

Patients with GP had a significantly higher mean age at Tx by 3.4 years (p = .002). The frequencies of post-Tx GP in the subgroups defined by < 50, 50–60, 61–70, and > 70-years-of-age at Tx were 4.8%, 6.4%, 9.5% and 11.4%, respectively.

5.2. Current state of SPE/SIFE screening

The current trends in SPE/SIFE screening in SOT recipients were assessed by calculating the percentage of patients who had undergone at least one SPE/SIFE test among all SOT recipients eligible for screening. Additionally, the average number of screenings per patient and the average time between post-Tx SPE/SIFE screenings for each Tx type were calculated.

From 2005 to 2015, SPE/SIFE analysis pre- and post-Tx was requested in 12.9% and 28.1% of the 3652 SOT recipients, respectively. On average, SOT recipients were screened 3.9 times with SPE/SIFE during the post-Tx follow-up period. The most frequently screened patients were liver transplant (LTx) patients (69.0%), with an average of 0.53 post-Tx screenings per year per patient. The results are shown in Table 3.

5.3. Clinical significance of gammopathies

The presence of GP in cohort A was associated with 6.06-fold (95% confidence interval 2.51–14.64) and 2.61-fold (1.49–4.6) higher hazard of PTLD and allograft loss, compared to those of SOT patients without GP. GP was also associated with a 1.99-fold (1.2–3.3) higher risk of death compared to that of the non-GP subgroup (Table 4).

Competing risks analysis showed similar results with subhazard ratios (95% confidence intervals) of 5.05 (1.98–12.9) and 2.35 (1.30–4.28) for PTLD and allograft loss, respectively (results not shown).

The observed 5-, 10-, 12- and 15-year survival rates of SOT recipients with GP were 93.7%, 83.7%, 65.7% and 31.0%, respectively. The median survival was 10.6 (IQR 5.1–13.3) years.

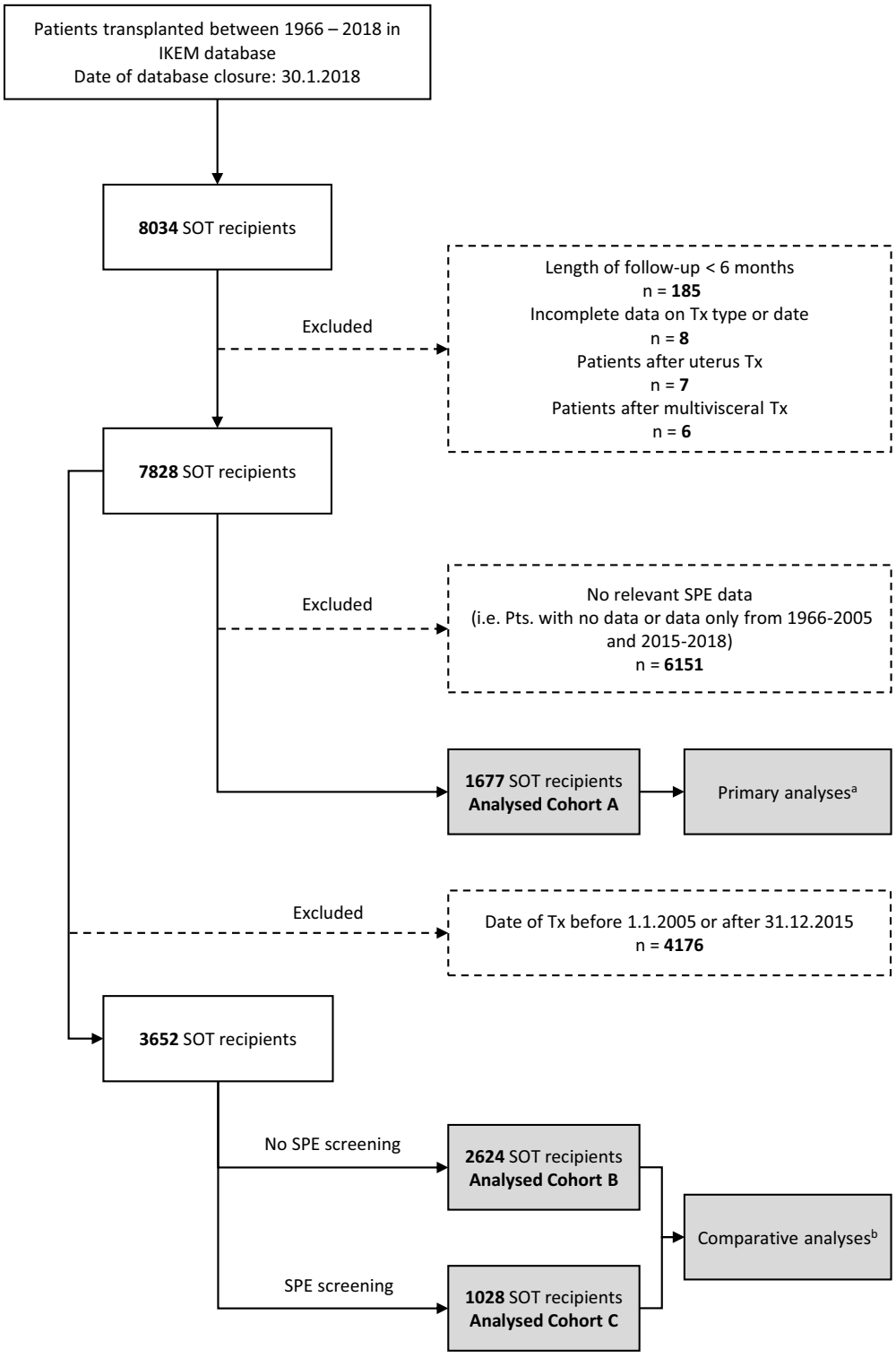
5.4. Diagnostic performance of SPE/SIFE in PTLD patients

Analysing post-Tx data, among the patients for whom an SPE/SIFE result was available within 2 years before the PTLD diagnosis, GP was detected in 4 of the 27 PTLD cases (Fig. 4).

The SPE/SIFE result was true-positive in all 3 cases of multiple myeloma and in 1 case of diffuse large B-cell lymphoma (DLBCL). In all other cases of lymphoproliferation (9 DLBCL, 2 T/NK cell, 1 Hodgkin, 1 Burkitt, 2 B-chronic lymphocytic leukaemia/small lymphocytic lymphoma, 1 mucosa-associated lymphatic tissue, 1 mantle-cell, 1 lymphoblastic diffuse, 1 plasmablastic, 3 unspecified B-cell non-Hodgkin and 1 unspecified lung lymphoma), the SPE/SIFE result was false-negative. The resulting diagnostic sensitivity and specificity were 14.8% and 93.9%, respectively (Table 5).

None of the 20 patients with detected pre-Tx GP subsequently developed PTLD.

Additional analysis comprised patients with and without SPE/IFE that was performed post-Tx. We observed that PTLD was diagnosed earlier (2.2 vs. 3.5 years) and more frequently (1.9 vs. 0.6%) if SPE/SIFE was included in post-Tx follow-up (Table 6).



[a] GP Frequency and time of first detection, Clinical significance of GPs (time-to-event analysis), Diagnostic performance of SPE/SIFE in PTLD diagnostics
[b] Current state of SPE/SIFE screening in SOT recipients, Efficiency of SPE/SIFE in PTLD diagnostics

GP = gammopathy, PTLD = posttransplantation lymphoproliferative disorder, SOT = solid organ transplantation, SPE/SIFE = serum protein electrophoresis / immunofixation, Tx = transplantation

Fig. 1. Patient recruitment flowchart.

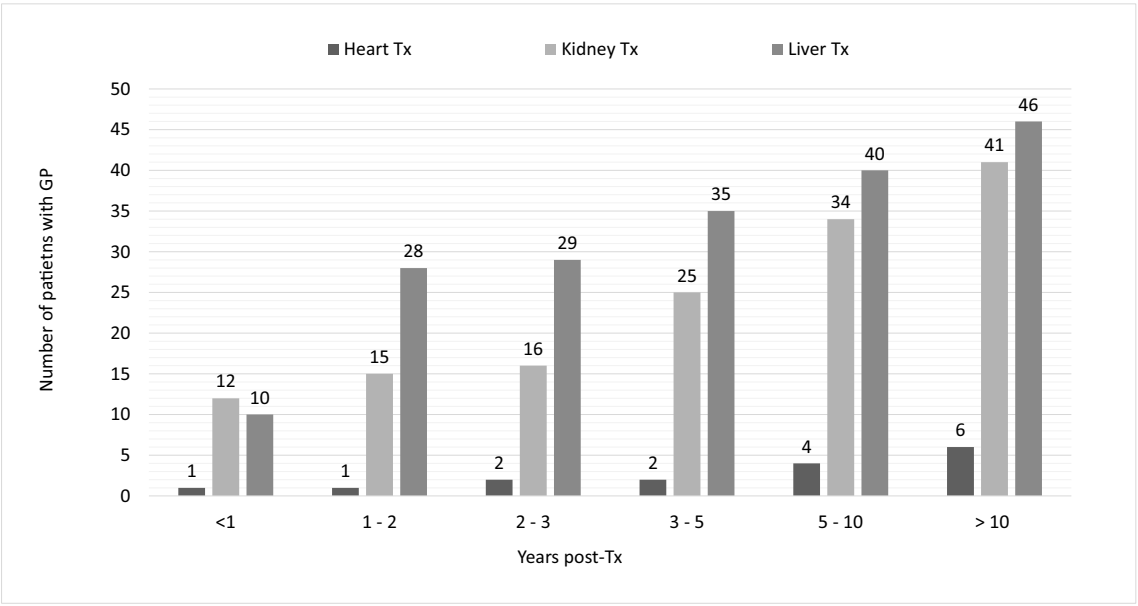


Fig. 2. GP distribution over follow-up period after various types of Tx. Incremental absolute counts of living patients with GP at given timepoint after Tx are shown (analysed patients: cohort A, $n = 1677$).

6. Discussion

6.1. Frequency of gammopathies

We found post-Tx GP in 6.4% of SOT recipients (Table 2), which is more frequent than the 3.2% prevalence reported in the general population [39]. The published data on GP incidence in the Tx population vary considerably, with 25–50% in HTx, 21–44% in LTx, and 0.6–25% in KTx patients. The frequency of GP increased with age from 4.8% in the group < 50-years-of-age to 11.4% in the group > 70-years-of-age at Tx. A similar trend was observed in the non-Tx [39] and Tx populations [21,23,30].

Monoclonal GP, particularly of the IgG isotype, was the most common type of GP, followed by oligoclonal and biclonal GP (Fig. 3). The proportion of the different types of GP was in line with previous reports [15] as well as data from the general population [39]. Interestingly, we found a high frequency of IgM GP (11%), which is reported to have the highest risk of subsequent PTLD [6].

The majority of GP were found in the first 3 years after Tx. The first detection of GP can be divided into three phases, displaying an initial rise in the first year, a plateau in years 2 and 3, followed by a steady rise in the next several years (Fig. 2). The observed trend was probably a combination of the effects of immunosuppressive regimens and age, and the trend was observed for KTx, LTx and HTx patients.

6.2. Clinical significance of gammopathies

Pre-Tx GP was described as having a limited relationship to post-Tx lymphoproliferation [5,18,24,30,35]. These data are consistent with our results, which found no PTLD in the pre-Tx GP group. On the other hand, pre-Tx GP appears to be linked to non-malignant complications, such as venous thrombosis and infection [35] as well as poor survival [30].

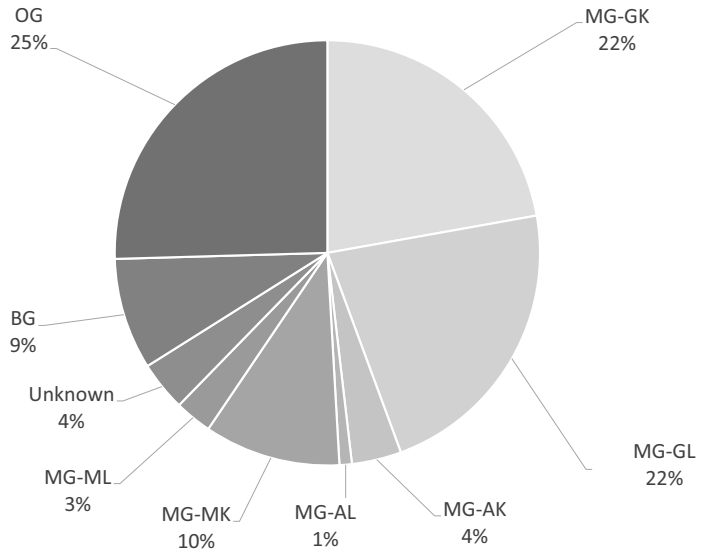
We believe that screening for GP should be included in the pre-Tx cancer workup of SOT recipients, especially the subgroup > 50-years-of-age. However, after ruling out plasma cell dyscrasia or the relationship of clonal immunoglobulin to organ failure (e.g., AL amyloidosis or light-chain deposition disease), pre-Tx GP should not be seen as a contraindication for SOT. Follow up for non-malignant complications is advisable in pre-Tx GP patients.

In contrast to pre-Tx GP, we found that post-Tx GP was a significant risk factor that was independent of age or sex for all tested effects in the Cox hazard model as well as Fine and Gray model. It is important to highlight that there is a large amount of conflicting data that has been

Table 2
Frequency of new cases and the time of the first detection of GP, following various types of Tx. Absolute and relative counts are shown for the frequency data. The time of the first detection is given as the median (IQR) years post-Tx (analysed patients: cohort A, n = 1677).

GP type	GP Frequency					GP first detection (years)	Persistent GP
	Heart Tx	Kidney Tx	Liver Tx	Combined Tx[a]	All Tx	All Tx	All Tx
Monoclonal	3 (7.7%)	37 (5.2%)	30 (3.6%)	1 (1.4%)	71 (4.3%)	2.0 (1.0–7.9)	76.4%
Biclonal	0	4 (0.6%)	3 (0.4%)	2 (2.7%)	9 (0.5%)	4.0 (2.8–9.3)	83.3%
Oligoclonal	3 (7.7%)	4 (0.6%)	16 (1.9%)	4 (5.4%)	27 (1.6%)	2.0 (1.0–6.1)	39.1%
All GP	6 (15.4%)	45 (6.4%)	49 (5.9%)	7 (9.5%)	107 (6.4%)	2.0 (1.0–7.0)	67%

[a] Kidney + Pancreas Tx, Liver + Kidney Tx.



OG = oligoclonal GP, BG = biclonal GP, MG = monoclonal GP, GK = IgG kappa, GL = IgG lambda, AK = IgA kappa, AL = IgA lambda, MK = IgM kappa, ML = IgM lambda

Fig. 3. Percentual proportion of different types of post-Tx GP (analysed patients: cohort A, $n = 1677$).

published to date. An association between GP and PTLD was previously reported in KTx, HTx and LTx recipients [3,5,6,21,34]. On the other hand, multiple publications reported opposing results [24–26,28,40]. In the present study, the observed post-Tx SPE/SIFE findings can be divided into 3 types.

The first type is the transient GP, comprising one third of all GP. These GP are present for a limited time, possibly representing immunosuppression or an infection-driven disbalance between T- and B-cell regulatory pathways [41]; and are probably of little clinical significance. In the present study, none of the transient GP was followed by subsequent PTLD implicating no connection to malignant lymphoproliferation.

The second type is the persistent GP associated with monoclonal gammopathy of undetermined significance (MGUS) and asymptomatic/smouldering multiple myeloma, which is the most common type (63%). These patients are at risk of subsequent PTLD, allograft loss or death, and should be risk-stratified [42,43] and managed accordingly [3,5,42,43]. We speculated, if relationship between GP and allograft loss could be explained by dysregulated immunoglobulin production ultimately causing humoral allograft rejection. Of particular interest is the strong association of GP and the loss of kidney allograft. This finding is in line with the monoclonal GP of renal significance observed in Tx [44] and non-Tx patients [45] and is consistent with the known ability of clonal immunoglobulins to cause kidney damage.

The third type is the persistent GP associated with plasma cell or other PTLD, which is the most clinically serious and the least common type (4%). Most of these patients had plasma cell dyscrasia requiring immediate diagnostic and therapeutic steps.

Table 4
Post-Tx GP as a risk factor for adverse events after Tx in Cox hazard model adjusted for age at Tx (analysed patients: cohort A, $n = 1677$).

Tx type[a]	Adverse effect (event)	Hazard ratio	95% CI	p
All SOT	PTLD	**6.06**	2.51–14.64	< 0.001
	Allograft loss	**2.61**	1.49–4.6	< 0.001
	Death	**1.99**	1.2–3.3	0.008
Kidney	Allograft loss	**2.95**	1.58–5.49	< 0.001
	Death	1.87	0.97–3.58	0.061
Liver	Allograft loss	1.93	0.26–14.37	0.520
	Death	1.99	0.80–4.94	0.137

Bold font is used to highlight statistically significant hazard ratios.
[a] Other combinations of Tx types and adverse effects contained too small numbers to analyse separately.

6.3. The use of blood serum-based methods for PTLD screening

There is still a need for a screening method to identify high-risk patients who might benefit from more PTLD-focused post-Tx care.

One of the options is monitoring EBV DNA-emia post-Tx. British

Table 3
Current state of SPE/SIFE screening in 3652 SOT recipients eligible for pre-Tx and post-Tx screening. Absolute and relative counts for 5 major Tx types are shown (analysed patients: cohort B, $n = 2624$; cohort C, $n = 1028$).

Screening characteristics	Tx type					
	Heart	Kidney	Liver	Kidney + Liver	Kidney + Pancreas	All Tx[a]
SPE/SIFE screening pre-Tx	6 (1.3%)	88 (4.2%)	359 (44.3%)	12 (37.5%)	5 (1.9%)	470 (12.9%)
SPE/SIFE screening post-Tx	23 (4.9%)	395 (19.0%)	559 (69.0%)	21 (65.6%)	30 (11.3%)	1028 (28.1%)
Post-Tx SPE/SIFE analyses per patient (average)	1.5	2.4	5.3	9.2	1.7	3.9
Number of post-Tx SPE/SIFE analyses per year and patient (average)	0.17	0.2	0.53	0.97	0.15	0.36

[a] Tx types with too small numbers to analyse separately were excluded (Pancreas, Liver + Heart, Heart + Lung, Multiple organ, Langerhans islets, Heart + Kidney, Kidney + Langerhans islets, Liver + Langerhans islets).

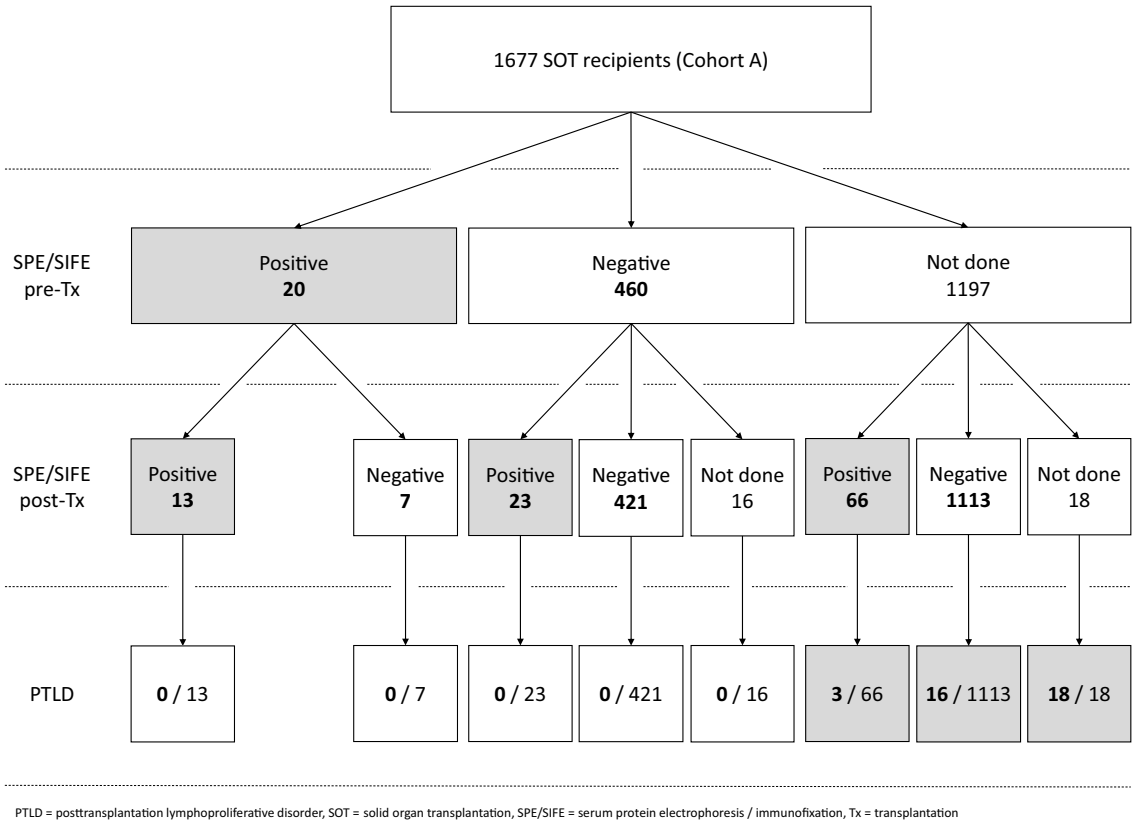


Fig. 4. Summary view of SPE/SIFE findings pre- and post-Tx in relation to PTLD diagnosis (analysed patients: cohort A, $n = 1677$).

Table 5
Diagnostic performance of SPE/SIFE for PTLD (analysed patients: cohort A, $n = 1677$).

Parameter	Value (%)	95% CI (%)
Sensitivity	14.8	4.2–33.7
Specificity	93.9	92.7–95.1
Positive predictive value	3.9	1.6–9.3
Negative predictive value	98.5	98.3–98.7
Accuracy	92.6	91.3–93.9

guidelines offer practical evidence-based recommendations that provide advice on the regular follow-up of patients after SOT, including EBV viral load monitoring and serology [2]. Patients with primary EBV infection after Tx should be always under more rigorous follow-up. However, the guidelines discourage the routine surveillance of EBV viral load in adult SOT recipients as a tool for PTLD screening (Recommendation Grade B, Level 3).

We have clearly demonstrated that GP is a significant risk factor for subsequent PTLD, with a 600% increase in the hazard for PTLD. The gold standard for the detection of GP is a combination of SPE and SIFE methods, primarily used as diagnostic and screening tools for plasma cell dyscrasias [43].

Based on this, we aimed to evaluate the diagnostic performance of SPE/SIFE in PTLD screening. This had proved rather complicated due to the inconsistent ordering of SPE/SIFE in SOT recipients and the resulting variable time intervals of SPE/SIFE results from the time of PTLD diagnosis. Thus, we included only SPE/SIFE results that were from within 2 years before the PTLD diagnosis.

In combination with the low PTLD prevalence, this led to a low number of patients with PTLD (27 of 47) who had relevant SPE/SIFE results. Thus, the input data are likely not optimal for this type of analysis; however, these results may be valuable, especially in light of other literature data missing (Table 5).

In our cohort, SPE/SIFE detected GP in 4 (14.8%) PTLD patients, which discourages from its use as a single, universal screening method. In previous studies, the presence of GP in PTLD was higher than observed in our cohort, varying between 50 and 86% [5,6,21,31,33]. An explanation might lie in the different study designs, with more frequent screening with SIFE [3,6,33] and urine immunofixation (UIFE) [6] or the systematic ½-1 year ordering of SPE in all patients [5]. On the other hand, most of the previous studies evaluated smaller number of PTLD cases and did not use diagnostically more relevant SPE results obtained within 2 years before lymphoproliferation diagnosis.

Apparently, the main pitfall in using GP detection methods for lymphoproliferation screening is that not all PTLDs are linked with clonal immunoglobulin production detectable by SPE/SIFE. Thus, due to the different nature and resulting diagnostic implications, it seems logical to divide PTLD patients into following subgroups.

Table 6
Effectiveness of PTLD diagnostics when SPE/SIFE was and was not included in the post-Tx screening (analysed patients: cohort B, $n = 2624$; cohort C, $n = 1028$).

Patient cohort	No. of patients	Diagnosed PTLD cases	Median (IQR) of Tx-PTLD diagnosis time
All Tx (B + C)	3652	37 (1.0%)	3.2 (1.3–4.7)
Tx with SPE/SIFE (C)	1028	20 (1.9%)	2.2 (1.1–4.1)
Tx without SPE/SIFE (B)	2624	17 (0.6%)	3.5 (1.3–5.4)

6.3.1. Plasma cell PTLD

The few studies that have focused on the subject suggest that plasma cell lymphoproliferations comprise 4% of all PTLDs and are mostly EBV negative [22,50–52].

In the present study, we identified only 3 cases of plasma cell PTLD among the available SPE/SIFE data. Promisingly, SPE/SIFE detected monoclonal GP in all 3 patients; however, SFLC and UIFE analysis was not performed. According to the literature, SPE/SIFE, SFLC and UIFE provide different insights, and some high-risk GP are detectable exclusively by only one of the methods [6,41,46–49,54].

Moreover, if we look closely at the different Tx populations at IKEM, HTx recipients are rarely (4.9%) screened for GP (Table 3). In contrast, these patients have the highest frequency of GP (15.4%), among all SOT (Table 2). This is in line with generally more aggressive immunosuppressive regimens compared to KTx or LTx patients.

Therefore, the combination of SPE/SIFE, SFLC and UIFE performed at regular intervals is effective for searching for MGUS and plasma cell PTLD; this strategy should be used without further hesitation for the screening of SOT recipients, with the highest potential benefit for HTx patients

6.3.2. Non-plasma cell PTLD

The most common PTLDs after SOT are non-Hodgkin lymphomas (NHL), particularly DLBCL, which are associated with an SPE-detectable GP in only 7% of cases [53]. One of the reasons might lie in the low sensitivities of SPE and SIFE to detect free light chain GP [47,54]. This issue might be solved by combining SPE/SIFE with high-sensitivity methods, such as SFLC and UIFE.

In the study performed by Martin on 208 NHL patients, 19 (9%) patients were only SFLC positive, 20 (10%) patients were only SPE/SIFE positive, and 7 (3%) patients had GP detectable by both methods [55]. In addition, Maurer reported the pathologic SFLC ratio in 41 (14%) of 295 analysed DLBCL cases [56].

SFLC was also tested for PTLD diagnostics, however, conflicting results were obtained. Engels found that elevated levels of SFLC are linked to subsequent PTLD [36], but this conclusion was not confirmed by Fernando [57]. Moreover, evaluating 169 SOT recipients, Kuhn concluded that the combination of SPE and SFLC is useful for PTLD screening [33].

Additionally, some monoclonal light chains can only be detected by UIFE [46,47,54]. Badley reported, that 42% of PTLDs had light-chain GP, which was detected by UIFE but not SPE/SIFE [6].

In summary, SPE/SIFE is not sensitive enough to be used as a single screening method for non-plasma cell PTLD. However, combined use with SFLC and UIFE may pinpoint patients at risk, trigger further evaluation and reduce the time to the diagnosis of lymphoproliferation.

6.4. Rationale for the proposed post-Tx algorithm

It is apparent that the transplant population at IKEM has very poor pre-Tx and post-Tx SPE/SIFE screening coverage (12.9% and 28.1%, respectively) and virtually no SFLC and UIFE coverage (Table 3). This is in sharp contrast with the observed clinical significance of GP findings post-Tx. To estimate the impact of adding SPE/SIFE to PTLD screening, we compared PTLD prevalence and time from Tx to PTLD diagnosis in SOT recipients with SPE/SIFE included and SPE/SIFE omitted from screening. The results appeared promising, as the use of SPE/SIFE seems to have increased the number of diagnosed cases of PTLD and to have shortened the time to PTLD diagnosis (Table 6).

Therefore, we propose using GP detection methods as first-line screening, along with clinical examination and other laboratory tests to identify early PTLD. Patients with known risk factors such as higher age, CMV/EBV infection, and immunosuppressive regimen using induction dose, T-cell depleting antibodies (anti-thymocyte globulin, anti-CD3 antibody Muromonab), and/or calcineurin inhibitors (ciclosporin, tacrolimus) require closer follow-up [2,7,13,14,21,23]. In the case of unexplained abnormalities in screening tests, patients should be referred to a hematologist, who will decide on the indication for second-line tests to rule out or confirm a PTLD diagnosis.

The combination of SPE (with subsequent SIFE for GP typing and confirmation), SFLC and UIFE is optimal for GP detection [46,47]. To separate transient and persistent GP, repeated analysis is needed. If the GP is progressive (a shift in clonality or an increase in concentration), referral to a hematologist is advisable. If no other signs of PTLD are detected, the monitoring of patients at regular intervals seems appropriate [5].

The frequency of further follow-up can be based on the MGUS risk stratification with the SPE/SIFE and SFLC results as input variables [42,43,46]. Monitoring for asymptomatic/smouldering multiple myeloma must be managed by hematologist to identify progression without delay [42,43]. The proposed algorithm is shown in Fig. 5.

7. Conclusions

GP after SOT, even without other symptoms, has to be considered a significant finding. GP should be searched for, and if found, managed accordingly. We found that GP is associated with a high risk of PTLD, allograft loss and poor survival and has a direct pathophysiological connection to post-Tx plasma cell dyscrasia.

It is important to emphasize that neither of the tests for GP detection has sufficient sensitivity to be used as a single-screening method for all PTLD patients. The best results are achieved when the tests are combined, and in plasma cell PTLD. These methods are not diagnostic; they aid in identifying patients who are at risk for PTLD and trigger further diagnostic processes. If used in parallel with targeted clinical examination and the EBV and CMV serostatus determination of donors and recipients, these tests can increase the effectiveness of PTLD diagnostics.

7.1. Limitations

The results might have been influenced by the retrospective nature of the study and the irregular screening with SPE. This most likely had the greatest effect on the detection of transient GP, which are present only for a short period of time. Persistent MGUS findings were likely to be detected even in cases of infrequent SPE screenings. Partial verification bias exists, because not all patients received equal surveillance for PTLD. Those with positive SPE were more likely to be examined more thoroughly (e.g. examination by hematologist, CT/MRI, bone-marrow biopsy etc.). However, all patients after SOT were under rigorous follow-up, although not with special regard to PTLD. Due to nature of the study, we cannot estimate the extent to which it has affected the results, but it is unlikely it has modified the observed associations. As bone marrow biopsy data were not available for all patients with persistent GP, we were unable to clearly separate MGUS and smouldering multiple myeloma. Urine studies or SFLC were not available to help identify light-chain GP.

7.2. Strengths

We analysed a large cohort of patients after multiple Tx types, with available SPE/SIFE and consistent clinical data during follow-up. A relatively high number of PTLD cases were included in comparison to similar studies. The standardized SPE/SIFE analysis and electrophoretogram interpretation minimized the inherently subjective nature of the method. The robust statistical evaluation of the clinical significance using the Cox proportional hazard model and Fine and Gray model for competing risks analysis ensured the validity of the observed results.

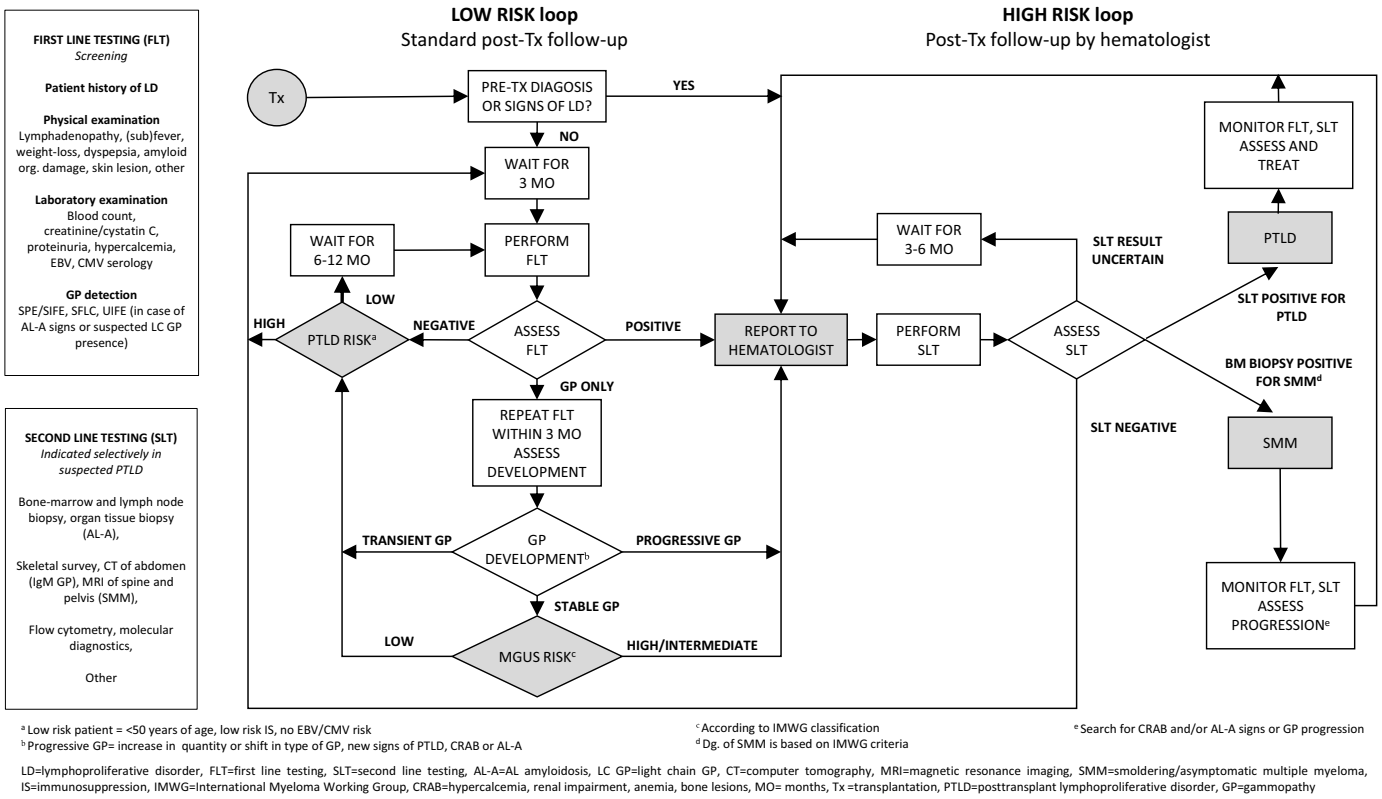


Fig. 5. Proposed PTLD screening algorithm.

Authorship

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Disclosure

Declaration of interests: none.

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References

[1] R. Bishnoi, R. Bajwa, A.J. Franke, et al., Post-transplant lymphoproliferative disorder (PTLD): single institutional experience of 141 patients, Exp. Hematol. Oncol. 6 (2017) 26.

[2] A. Parker, K. Bowles, J.A. Bradley, et al., Diagnosis of post-transplant lymphoproliferative disorder in solid organ transplant recipients – BCSH and BTS guidelines, Br. J. Haematol. 5 (2010) 675–692.

[3] D.E. Tsai, N.A. Aqui, J.E. Tomaszewski, et al., Serum protein electrophoresis abnormalities in adult solid organ transplant patients with post-transplant lymphoproliferative disorder, Clin. Transpl. 5 (2005) 644–652.

[4] J.L. Koff, J.X. Li, X. Zhang, et al., Impact of the posttransplant lymphoproliferative disorder subtype on survival, Cancer. 124 (2018) 2327–2336.

[5] A. Lemoine, P. Pham, D. Azoulay, et al., Detection of gammopathy by serum protein electrophoresis for predicting and managing therapy of lymphoproliferative disorder in 911 recipients of liver transplants, Blood. 5 (2001) 1332–1338.

[6] A.D. Badley, D.F. Portela, R. Patel, et al., Development of monoclonal gammopathy precedes the development of Epstein-Barr virus-induced posttransplant lymphoproliferative disorder, Liver Transpl. Surg. 5 (1996) 375–382.

[7] I.P. Neuringer, Posttransplant lymphoproliferative disease after lung transplantation, Clin. Dev. Immunol. 2013 (2013) 430209.

[8] G. Opelz, B. Doehler, Pediatric kidney transplantation: analysis of donor age, HLA match, and posttransplant non-Hodgkin lymphoma: a collaborative transplant study report, Transplantation. 3 (2010) 292–297.

[9] J. Morscio, T. Tousseyn, Recent insights in the pathogenesis of post-transplantation lymphoproliferative disorders, World J. Transplant. 3 (2016) 505–516.

[10] S. Choquet, S. Varnous, C. Deback, et al., Adapted treatment of Epstein-Barr virus infection to prevent posttransplant lymphoproliferative disorder after he art transplantation, Am. J. Transplant. 4 (2014) 857–866.

[11] U. Allen, J. Preiksaitis, AST Infectious Diseases Community of Practice. Epstein-Barr virus and posttransplant lymphoproliferative disorder in solid organ transplant recipients, Am. J. Transplant. (9 Suppl 4) (2009) S87–S96.

[12] J. Cheng, C.A. Moore, C.J. Iasella, et al., Systematic review and meta-analysis of post-transplant lymphoproliferative disorder in lung transplant recipients, Clin. Transpl. 32 (2018) e13235.

[13] I. Doycheva, S. Amer, K.D. Watt, De novo malignancies after transplantation. Risk and surveillance strategies, Med. Clin. N. Am. 100 (2016) 551–567.

[14] S.C. Quinlan, R.M. Pfeiffer, L.M. Morton, et al., Risk factors for early-onset and late-onset post-transplant lymphoproliferative disorder in kidney recipients in the United States, Am. J. Hematol. 86 (2011) 206–209.

[15] G. Alfano, F. Fontana, E. Colaci, et al., Monoclonal gammopathy of undetermined significance after kidney transplantation: single-center experience, Transplantation. 11 (2017) e337–e342.

[16] J. Passweg, G. Thiel, H.A. Bock, Monoclonal gammopathy after intense induction

immunosuppression in renal transplant patients, Nephrol. Dial. Transplant. 12 (1996) 2461–2465.
[17] I. Myara, G. Quenum, M. Storogenko, et al., Monoclonal and oligoclonal gammopathies in heart-transplant recipients, Clin. Chem. 8 (1991) 1334–1337.
[18] H.V. Naina, S. Harris, A. Dispenzieri, et al., Long-term follow-up of patients with monoclonal gammopathy of undetermined significance after kidney transplantation, Am. J. Nephrol. 4 (2012) 365–371.
[19] E.A. Engels, B. Savoldo, R.M. Pfeiffer, et al., Plasma markers of B-cell activation and clonality in pediatric liver and hematopoietic stem cell transplant recipients, Transplantation. 3 (2013) 519–526.
[20] N. Babel, F. Schwarzmann, A. Pruss, et al., Monoclonal gammopathy of undetermined significance (MGUS) is associated with an increased frequency of Epstein-Barr Virus (EBV) latently infected B lymphocytes in long-term renal transplant patients, Transplant. Proc. 9 (2004) 2679–2682.
[21] A.L. Caforio, A. Gambino, A. Belloni Fortina, et al., Monoclonal gammopathy in heart transplantation: risk factor analysis and relevance of immunosuppressive load, Transplant. Proc. (1–2) (2001) 1583–1584.
[22] X. Sun, L.C. Peterson, Y. Gong, et al., Post-transplant plasma cell myeloma and polymorphic lymphoproliferative disorder with monoclonal serum protein occurring in solid organ transplant recipients, Mod. Pathol. 4 (2004) 389–394.
[23] C. Chakalarovski, P. Lang, C. Buisson, et al., Monoclonal immunoglobulins in patients with renal transplants: characterization, evolution and risk factors, Transpl. Int. 5 (Suppl. 1) (1992) S23–S25.
[24] V.H. Jimenez-Zepeda, R.L. Heilman, R.A. Engel, et al., Monoclonal gammopathy of undetermined significance does not affect outcomes in patients undergoing solid organ transplants, Transplantation. 5 (2011) 570–574.
[25] I. Bancu, L. Cañas, F.J. Juega, et al., Outcomes of monoclonal gammopathy of undetermined significance in patients who underwent kidney transplantation, Transplant. Proc. 8 (2015) 2344–2345.
[26] C. Cuéllar-García, C. Sevillano Ruiz-Mateos, M.A. Mazuecos Blanca, et al., Follow-up monoclonal gammopathy of undetermined significance in kidney transplant, Transplant. Proc. 1 (2015) 78–80.
[27] D. Peest, B. Schaper, B. Nashan, et al., High incidence of monoclonal immunoglobulins in patients after liver or heart transplantation, Transplantation. 3 (1988) 389–393.
[28] G. Touchard, T. Pasdeloup, J. Parpeix, et al., High prevalence and usual persistence of serum monoclonal immunoglobulins evidenced by sensitive methods in renal transplant recipients, Nephrol. Dial. Transplant. 6 (1997) 1199–1203.
[29] R.R. Redfield, A. Naji, Progression of monoclonal gammopathy of undetermined significance to multiple myeloma in a solid organ transplant, Transplantation. 12 (2011) e65–e66 author reply e66.
[30] L. Soltero, H. Carbajal, J. Xu, et al., Initial survival data of kidney transplant patients with pre-transplant monoclonal gammopathy, Clin. Transpl. 2 (2012) 300–304.
[31] D.E. Tsai, N.A. Aqui, J.E. Tomaszewski, et al., Serum protein electrophoresis abnormalities in adult solid organ transplant patients with post-transplant lymphoproliferative disorder, Clin. Transpl. 5 (2005) 644–652.
[32] S. Safadi, A. Dispenzieri, H. Amer, et al., Multiple myeloma after kidney transplantation, Clin. Transpl. 1 (2015) 76–84.
[33] D. Kuhn, P. Wang, I. Shu, et al., Comparison of free light chain assay with protein electrophoresis for screening and monitoring PTLD, Blood. 124 (2014) 5883.
[34] G.P. Pageaux, A. Bonnardet, M.C. Picot, et al., Prevalence of monoclonal immunoglobulins after liver transplantation: relationship with posttransplant lymphoproliferative disorders, Transplantation. 3 (1998) 397–400.
[35] T.E. Goebel, N.K. Schiltz, K.J. Woodside, et al., Neoplastic and non-neoplastic complications of solid organ transplantation in patients with preexisting monoclonal gammopathy of undetermined significance, Clin. Transpl. 9 (2015) 851–857.
[36] J. Tate, G. Caldwell, J. Daly, et al., Recommendations for standardized reporting of protein electrophoresis in Australia and New Zealand, Ann. Clin. Biochem. 3 (2012) 242–256.
[37] S.H. Swerdlow, E. Campo, S.A. Pileri, et al., The 2016 revision of the World Health Organization classification of lymphoid neoplasms, Blood. 20 (2016) 2375–2390.
[38] J.P. Fine, R.J. Gray, A proportional hazards model for the subdistribution of a competing risk, J. Am. Stat. Assoc. 446 (1999) 496–509.
[39] R.A. Kyle, T.M. Therneau, S.V. Rajkumar, et al., Prevalence of monoclonal gammopathy of undetermined significance, N. Engl. J. Med. 13 (2006) 1362–1369.
[40] G. Alfano, F. Fontana, E. Colaci, et al., Immunophenotypic profile and clinical outcome of monoclonal B-cell lymphocytosis in kidney transplantation, Clin. Transpl. 8 (2018) e13338.
[41] E.A. Engels, J. Preiksaitis, A. Zingone, et al., Circulating antibody free light chains and risk of posttransplant lymphoproliferative disorder, Am. J. Transplant. 5 (2012) 1268–1274.
[42] R.A. Kyle, B.G. Durie, S.V. Rajkumar, et al., Monoclonal gammopathy of undetermined significance (MGUS) and smoldering (asymptomatic) multiple myeloma: IMWG consensus perspectives risk factors for progression and guidelines for monitoring and management, Leukemia. 6 (2010) 1121–1127.
[43] S.V. Rajkumar, M.A. Dimopoulos, A. Palumbo, et al., International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma, Lancet Oncol. 12 (2014) e538–e548.
[44] A. Galioto, F. Morando, S. Rosi, et al., Monoclonal gammopathy after liver transplantation: a risk factor for long-term medical complications other than malignancies, Transpl. Int. 1 (2012) 25–33.
[45] N. Leung, F. Bridoux, C.A. Hutchison, et al., Monoclonal gammopathy of renal significance: when MGUS is no longer undetermined or insignificant, Blood. 22 (2012) 4292–4295.
[46] A. Dispenzieri, R. Kyle, G. Merlini, et al., International Myeloma Working Group guidelines for serum-free light chain analysis in multiple myeloma and related disorders, Leukemia. 2 (2009) 215–224.
[47] J.A. Katzmann, R.A. Kyle, J. Benson, et al., Screening panels for detection of monoclonal gammopathies, Clin. Chem. 8 (2009) 1517–1522.
[48] S.V. Rajkumar, R.A. Kyle, T.M. Therneau, et al., Serum free light chain ratio is an independent risk factor for progression in monoclonal gammopathy of undetermined significance, Blood. 3 (2005) 812–817.
[49] P. Sečník Jr., P. Lavríková, L. Hošková, et al., Free light chain and intact immunoglobulin abnormalities in heart transplant recipients: two year follow-up timelines and clinical correlations, Transpl. Immunol. 41 (2017) 22–26.
[50] R. Trappe, H. Zimmermann, S. Fink, et al., Plasmacytoma-like post-transplant lymphoproliferative disorder, a rare subtype of monomorphic B-cell post-transplant lymphoproliferation, is associated with a favorable outcome in localized as well as in advanced disease: a prospective analysis of 8 cases, Haematologica. 7 (2011) 1067–1071.
[51] M.A. Nalesnik, Plasma cell tumors in transplant patients, Blood. 8 (2013) 1247–1249.
[52] M. Karuturi, N. Shah, D. Frank, et al., Plasmacytic post-transplant lymphoproliferative disorder: a case series of nine patients, Transpl. Int. 6 (2013) 616–622.
[53] K.M. Charafeddine, M.N. Jabbour, R.H. Kadi, et al., Extended use of serum free light chain as a biomarker in lymphoproliferative disorders: a comprehensive review, Am. J. Clin. Pathol. 6 (2012) 890–897.
[54] G. Palladini, P. Russo, T. Bosoni, et al., Identification of amyloidogenic light chains requires the combination of serum-free light chain assay with immunofixation of serum and urine, Clin. Chem. 3 (2009) 499–504.
[55] W. Martin, R. Abraham, T. Shanafelt, et al., Serum-free light chain-a new biomarker for patients with B-cell non-Hodgkin lymphoma and chronic lymphocytic leukemia, Transl. Res. 4 (2007) 231–235.
[56] M.J. Maurer, I.N. Micallef, J.R. Cerhan, et al., Elevated serum free light chains are associated with event-free and overall survival in two independent cohorts of patients with diffuse large B-cell lymphoma, J. Clin. Oncol. 12 (2011) 1620–1626.
[57] R.C. Fernando, E.G. Rizzatti, W.M. Braga, et al., Serum free light chains and post-transplant lymphoproliferative disorder in patients with renal transplant, Leuk. Lymphoma 10 (2013) 2177–2180.