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Polymorphonuclear leukocyte functions as predictive markers for infections after organ transplantation

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Abstract Infectious complications are still a major cause of morbidity and mortality after organ transplantation, and early therapy would certainly reduce the risk associated with severe infections. We therefore investigated the significance of polymorphonuclear leukocyte (PMN) functional tests as predictive markers for infection in transplant patients under immunosuppressive therapy in a longitudinal study. In 41 patients, blood PMN migration and reactive oxygen species release, the blood levels of PMN elastase, malondialdehyde, neopterin, sICAM-1 and sVCAM-1, and urine neopterine were measured in 3- and 4-day intervals after liver-, kidney-, kidney-pancreas-, and heart and lung transplantation. PMN migration was determined in whole blood and estimated by the amount of PMNs to penetrate into a membrane filter upon FMLP stimulation. Three groups of patients were formed according to their postoperative course. Group I patients ($n = 23$) had no or only minor local infection, group II patients ($n = 11$) had infections with distinct systemic involvement, and group III patients ($n = 7$) developed sepsis. A first elastase-level of over 100 mg/L after surgery, followed by a drop in the amount of blood PMNs ready to migrate, on FMLP stimulation, to below 12%, turned out to be a marker for impending infection, whereas all other parameters tested were not predictive. In six of seven group III patients, this marker became positive (sensitivity 85.7%) up to 15 days before clinical manifestation of sepsis. In group I (largely uneventful recovery) only one of 23 patients was positive (specificity 95.6%), whereas group II patients were in between (4 of 11 positive). By this method it seems possible to diagnose severe infections in the preclinical phase, which may help prevent them if treatment is begun promptly.

Key words Transplantation · Sepsis · Infection · PMN · Predictive marker

Abbreviations *LTX* Liver transplant · *KPTX* Kidney-pancreas transplant · *KTX* Kidney transplant · *HTX* Heart transplant · *LKTX* Liver-kidney transplant · *LUTX* Lung transplant · *PMN* Polymormphonuclear leukocyte · *TMI* Total migration index · *DC* Distribution characteristic · *FMLP* N-formyl-methionyl-leucyl-phenylalanine · *ROS* Reactive oxygen species · *MDA* Malondialdehyde · *ABI* Antibiotic, antibiotics · *LOC* Without or with minor local infections · *SYS* Infections with systemic involvement, not septic · *SEP* Infections, septic

Introduction

New immunosupressive agents such as cyclosporin, tacrolimus or mycophenolat mofetil made prevention and treatment of acute rejection for the majority of allograft recipients possible. Unfortunately, their immunosuppressive effect is rather unspecific and also affects defence mechanisms. In consequence, infections are the most frequent complication associated with organ transplantation [3, 25] To the present there is no way to assess the degree of immunosuppression as well as the integrity or impairment of host defence mechanisms, and thus to assess the risk of infection.

In a pilot study we have been able to demonstrate that polymorphonuclear leukocyte (PMN) migration is impaired before sepsis appears [10]. PMNs are the foremost defence line against bacterial and fungal pathogens, and deficiencies in PMN functions create a hotbed for the development of infections [4, 5].Monitoring the signals of impending immune deficiency would offer an opportunity to prevent clinical manifestation of infections by early intervention. The present investigation is a further development of our former pilot study applying a more comprehensive protocol. Beside migration, several other PMN-related inflammatory parameters were measured, and their significance as predictive markers for infections was determined

Materials and methods

A total of 30 male and 11 female recipients at a mean age of 48.9 (25–70) years were examined. The transplanted organs were 22 livers (LTX), 9 kidney-pancreas (KPTX), 5 kidneys (KTX), 3 hearts (HTX), 1 liver-kidney (LKTX), 1 lung (LUTX). Routinely, the LTX, HTX, KPTX, and LUTX patient underwent perioperative antibiotic (ABI) prophylaxis with Piperacillin Tazobactam (Tazonam, Cyanamid, Wolfratshausen, Germany) for 3 days, the KTX-patients with Flucloxacillin (Floxapen, Smith Kline Beecham, Heppignies, Belgium) and Penicillin G (Biochemie, Wien, Austria) for 1 day. Immunosuppression was carried out with a triple drug regime comprising methylprednisolone (Urbason, Hoechst Marion Roussel, Frankfurt, Germany), starting with 500 mg during surgery, rapidly tapered to 25 mg, tacrolimus (Prograf, Fujisawa, Osaka, Japan) or cyclosporin (Cyclosporin A, Neoral, Novartis, Vienna, Austria), and azathioprine (Imurek, Glaxo Wellcome, Vienna, Austria) or mycophenolat mofetil (Cellcept, Roche, Basel, Switzerland). HTX and LTX patients underwent an induction therapy with ATG (Biomerieux, Vienna, Austria) 2–3 mg/kg BW for 5–10 days.

Bacterial infections and sepsis were treated empirically and later, according to sensitivity testing, with various antibiotics. CMV infections were treated with ganciclovir (Cymevene, Roche, Basel, Switzerland), and HSV infections with famvir (Famcyclovir, Smith Kline Beecham, Heppignies, Belgium). Rejection episodes were treated with methylprednisolone 3 × 500 mg for 3 days, and steroid resistant rejections with ATG as above for 7–10 days.

Beginning 2, 3, or 4 days after transplantation, several inflammatory parameters were examined in blood samples drawn from a central venous line or a cubital vein in NH4 heparin syringes (SARSTEDT, Nümbrecht, Germany). Informed consent was obtained. Examinations were carried out twice a week – in most cases in alternating 3–4 day intervals – until discharge. The duration of hospitalization (see Fig. 7), and consequently the number of examinations, was different for each patient. For more details, see the legends. All together 377 checks of each parameter were made.

Inflammation parameters

PMN functional parameters

The white blood count was carried out in a COULTER COUNTER T-540. PMN elastase was measured with the PMN elastase 1.12589 kit, (Merck, Vienna, Austria). PMN migration was measured by a novel membrane filter migration chamber, the main feature of which is the use of diluted, fresh, whole blood. Briefly, blood anticoagulized with ammonia-heparine was processed within 20 min after withdrawal [11]. The migration device includes a migration filter (cellulose nitrate membrane filter, 3 μm pore size, 140 μm thick, SARTORIUS, Göttingen, Germany) and a chemoattractant depot containing the bacterial chemotactic peptide N-formyl-methionyl-leucyl-phenylalanine (FMLP) in solid form that dissolves in the blood suspension pipetted into the chamber and forms a chemotactic gradient within the migration filter. The number of PMNs to penetrate into the filter is counted in consecutive layers of 10 μm throughout the whole depth of a filter. Such a count yields both the number of PMN immigrants and their distribution within a filter. Two relative parameters were considered in this study: a) the percentage of PMNs to migrate from the blood into the filter, the "total migration index", TMI, and b) the relative penetration depth, named "distribution characteristic", DC, expressed as the distance that half the migratory PMN bulk has covered within a filter. Both parameters were established for the reaction to FMLP and the blanks.

The TMI and the DC "quotients" are the relation between FMLP and the blank value, calculated for each pair of measurements. They express the reactivity of a PMN collective to FMLP in comparison with the unstimulated, spontaneous migration. For more details about the method see [8].

Following Kukovetz et al. [23], the latex-stimulated reactive oxygen species (ROS) release from phagocytes was measured at 22 °C in ammonia heparine-anticoagulated fresh whole blood [11] with luminol-enhanced chemilumenescence in a 1450 Microbeta luminometer (Wallace, Turku, Finland). During the first 30 min, measurements were carried out in 2-minute intervals, and, during the following 60 min, in 10-min intervals. The highest level measured in this time frame was regarded as relevant and applied to 1000 phagocytes, (PMNs, eosinophils, and monocytes). This value expresses the average oxidative efficiency of a phagocyte. Eosinophils can produce more, and monocytes less ROS than PMNs [14, 21]. Thus, a compensatory effect and the low share of eosinophils and monocytes in comparison with blood PMNs keep the bias within tolerable bounds, and the level can be applied to 1000 PMNs with sufficient accuracy.

Lymphocytes lacking NADPH-oxidase can be neglected [5]. Malondialdehyde (MDA), an indicator for oxidative membrane damage [12, 13] was measured with the Bioxytech LPO-586 kit, (Oxis, Portland, OR, USA).

Monocyte and endothelium functional parameters

Neopterin, an indicator for macrophage activation, was measured in urine by an HPLC-method [15], sICAM-1, a soluble endothelial ligand for PMN integrins, and sVCAM-1, a soluble endothelial ligand for monocyte and lymphocyte adhesins (reviewed by [6,

20]), were both measured with the ICAM and VCAM ELISA, R&D Systems (MN, USA). PMN elastase, MDA, sICAM-1 and sVCAM-1 were measured in EDTA plasma, harvested within 1 h after puncture from blood cooled to 4 °C. The plasma samples were stored at –80 °C until measurements were carried out.

Retrospectively, three groups of patients were formed according to the occurrence and the severity of infection.

Group I patients ($n = 23$) developed no or only moderate local infections (colonisation of the urinary tract, superficial wound infections) without clinically apparent systemic involvement. Some bacteriograms revealed *E.Coli, Klebsiella, Morganella spec., Enterobacter, Pseudomonas*, and coagulase negative *Staphylococci.* Treatment, if any, was local. No systemic ABI were applied.

Group II patients ($n = 11$) suffered from infections with impairment of clinical condition, systemic involvement such as rise in temperature and/or organ dysfunction. Positive bacteriograms *(E.Coli, Acinetobacter, Klebsiella, Enterococci, Enterobacter, Staph. aureus, Pneumococci, Citrobacter,* coagulase-negative *Staphylococci,* beta-hemolysing *Streptococci)* and, in the case of pneumonia, positive X-ray findings characterized these patients. Relevant infections of the urinary tract, bacteria cultured from bile or sputum, pneumonia and deep wound infections were predominant in this group. All of these patients were treated with systemic ABI.

Group III patients ($n = 7$) showed the clinical symptoms of sepsis that was confirmed by positive blood culture. Coagulase-negative *Staphylococci, Staph. aureus, Klebsiella, Pseudomonas* and *E. coli* were cultured. Similar types of infections as in group II were observed in these patients. All septic patients were treated with systemic ABI.

Infections with CMV and HSV were not considered in this classification, since they are known not to cause significant PMN reactions.

In order to estimate the inflammatory reactions of the three groups of patients by eye, the individual ratings were presented in scatter plots as follows: The ratings of group I were plotted in a single column. The ratings of groups II and III were divided into two columns each, the left column representing the reactions before, and the right column the reactions after onset of infection. The onset of an infection was defined as the occurrence of clear clinical symptoms and radiological signs as described above. These symptoms were also the indication for starting a systemic ABI therapy. Differences in the inflammation parameters in group I (no/local infections) and groups II and III (generalized infections) before the clinical manifestation of an infection were considered predictive indicators for a future infection. The limits distinguishing an uneventful course from future infectious complications were established empirically and were chosen to achieve a minimal error rate. They are indicated in the Figures by a line.

Tables 1–3 demonstrate how far the discriminative criteria determined collectively in the Figs. 1, 2, 3 and 5 are true for each individual patient. The tables comprise the parameters found to be predictive: elastase and the TMI under FMLP stimulation (TMI-FMLP), assisted by the FMLP/blank quotients of the TMI and the PMN blood count. Moreover, the latency period between surgery and the first measurement of a TMI-FMLP below the critical mark of "12", indicating the onset of an immune deficiency, is shown. In the case of infection, the time between surgery and the first manifestation of an infection, as well as the interval between the critical TMI-FMLP and the outbreak of an infection, are presented (Table 2, Table 3). Rejection episodes are listed individually.

Correlations between PMN blood counts and the appropriate TMI-FMLP values were calculated with the Spearman rank correlation test. The proportion of non-predictive to predictive for the groups LOC, SYS B and SEP B were compared with Fishers Exact Test contingency tables. For more details see the legends to Figs. 1–3, and 5. The proportions of predictive markers (positive elastase values in combination with positive TMI-FMLP values) to non predictive markers in the Tables 1, 2, and 3 were compared with Fisher's Exact Test contingency tables. The columns LOC, SYS and SEP shown in Fig. 7 (days of hospitalization) were compared with a Kruskal-Wallis ANOVA.

Significance symbols: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, n.s. not significant.

Table 1 Group I, patients without or with minor local infections (LOC). *Pat* Serial number of the patients, *EL* PMN elastase; positive: values above 100 µg/L, *TMI* total migration indices under FMLP stimulation (TMI-FMLP); positive: values below 12, occurring at least once, *Q* individual FMLP/blank quotients of the TMI; positive: values below 0.4, *PMN* PMN blood counts; positive: values above 15.000/µl, *REJ, R* patients with rejections, *IDE* appearance of immunodeficiency, i.e. the interval in days between transplantation and the first occurrence of a TMI-FMLP below 12, *TX* transplanted organs, *LTX* liver, *KTX* kidney, *KPTX* kidney and pancreas, *HTX* heart, *LUTX* lung

Pat	EL	TMI	Q	PMN	REJ	IDE	TX
1	–	–	–	–	R		LTX
2	–	–	–	–	R		KTX
3	–	–	–	–			LTX
4	–	–	–	–			KPTX
5	–	–	–	–			HTX
6	–	–	–	–			KTX
7	–	–	–	–			LUTX
8	–	–	–	–			LTX
9	–	–	–	–			LTX
10	–	–	–	–			KTX
11	–	–	–	–			LTX
12	–	–	–	–			KPTX
13	–	–	–	–			KTX
14	–	–	–	–			LTX
15	–	–	–	+			LTX
16	+	–	–	–			LTX
17	+	–	–	–	R		LTX
18	+	–	–	–	R		KPTX
19	+	–	–	–			HTX
20	+	–	–	+	R		LTX
21	–	+	–	–	R	3	LTX
22	–	+	+	–		3	KPTX
23	+	+	–	–		4	KPTX

Results

PMN blood count

(Fig. 1, Tables 1, 2, and 3). When setting a discriminative upper threshold of 15.000 PMNs, in group I 2 of 23 patients (one of them twice) were found to be above this limit. In group II, 1 of 11 patients, and in group III, 3 patients (in all seven times) were found to be above the limit. No patient in groups II and III was under the lower limit of 1000 PMN/µl usually considered to be the minimum for the functioning of the unspecific defence.

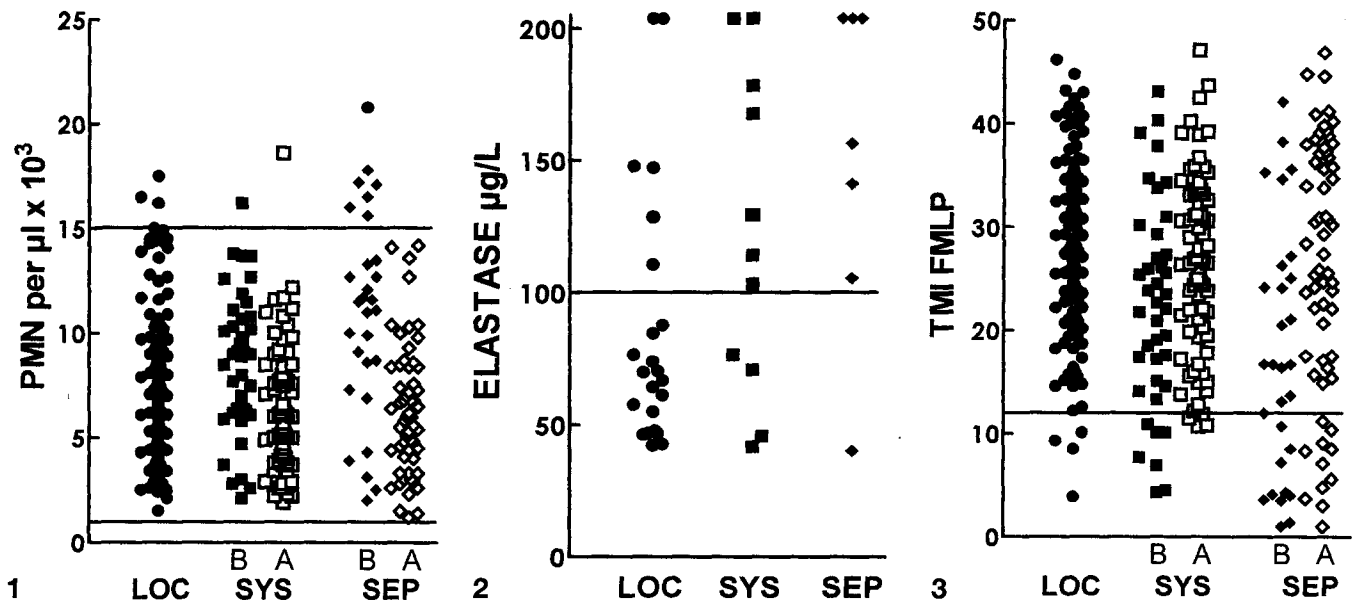

Fig. 1 PMN blood counts. LOC: Group I, no or local infections without systemic involvement, $n = 23$. SYS: Group II, infections with systemic involvement, $n = 11$. SEP: Group III, patients who developed sepsis $n = 7$. *Left columns* "B" with full symbols: levels measured before onset of infections. *Right columns* "A" with empty symbols measured after onset of infection. Tests were carried out twice a week. The duration of hospitalization of the individual patients is shown in Fig. 7. Thus, according to their stay in the clinic, the patients were subjected to different numbers of checks. The lower line marks the 1000 PMN threshold commonly considered to be the lower limit of an intact unspecific defence system. Note that no patient remained under this critical level. Quite in contrast to the current conception of an "immunodeficiency", several septic patients had markedly elevated PMN-levels before onset of infection. The discrimination limit for "elevated" was chosen at 15.000/µl blood. The proportions between "not elevated" (below 15.000 µl) and "elevated" (above 15.000) are for LOC 158:3, SYS B 39:1, and for SEP B 22:7. Contingency tables: LOC-SYS B n.s., LOC-SEP B ***, SYS B-SEP B **

Fig. 2 Elastase blood levels, first measurement after transplantation. LOC: Group I, no infection or local infections without systemic involvement. SYS: Group II, infections with systemic involvement. SEP: Group III, patients with sepsis. The upper methodical limit of the measurements was 204 µg/l. The discrimination limit is 100 µ/l. The proportions between "not predictive" (below 100 µg/l) and "predictive" (above 100) are for LOC 17:6, for SYS 4:7, and for SEP 1:6. Contingency tables: LOC-SYS n.s., LOC-SEP**, SYS-SEP n.s.

Fig. 3 PMN migration. The total migration index under FMLP stimulation (TMI-FMLP). Symbols as in Fig. 1. Values below the limit of 12 before onset of inflammation are considered to be predictive. The proportions between "not predictive" (above 12) and "predictive" (below 12) are for LOC 157:4, for SYS B 33:7, and for SEP B 19:10. Contingency tables: LOC-SYS**, LOC-SEP***, SYS-SEP n.s.

Elastase blood levels

Figure 2 and Tables 1, 2 and 3 show the ratings of the first measurement after surgery that proved to be indicative of further inflammatory events. This first measurement was always carried out before the manifestation of infection, usually 1, 2, and 3 days after transplantation. Later measurements were without predictive value and are therefore not presented. In group I, six (26%), in group II, seven (63.6%) and in group III, six patients (85.7%) had post-surgical elastase values above the discriminative mark of 100 µ/l.

PMN migration

For the TMI under FMLP stimulation (TMI-FMLP), a critical lower limit of "12" was set. Before clinical manifestation of an infection, the TMI-FMLP level dropped under this limit in three patients of group I (13%), in five of group II (45.4%), and in six of group III (85.7%). Some of these patients showed the drop repeatedly (Fig. 3, Tables 1, 2 and 3). The blanks (Fig. 4) did not develop discriminative differences. The FMLP to blank quotient (Fig. 5) was twice below the critical limit set at "0.4" in one patient in group I (4.3%), in four patients of group II (36.3%), and in four patients of group III (57.1%). As an example, Fig. 6 shows the time profile of a septic patient (number 39 in Table 3). PMN blood-counts and the migration parameter TMI-FMLP do not correlate (spearman $r = 0.1245$) and thus are independent of each other.

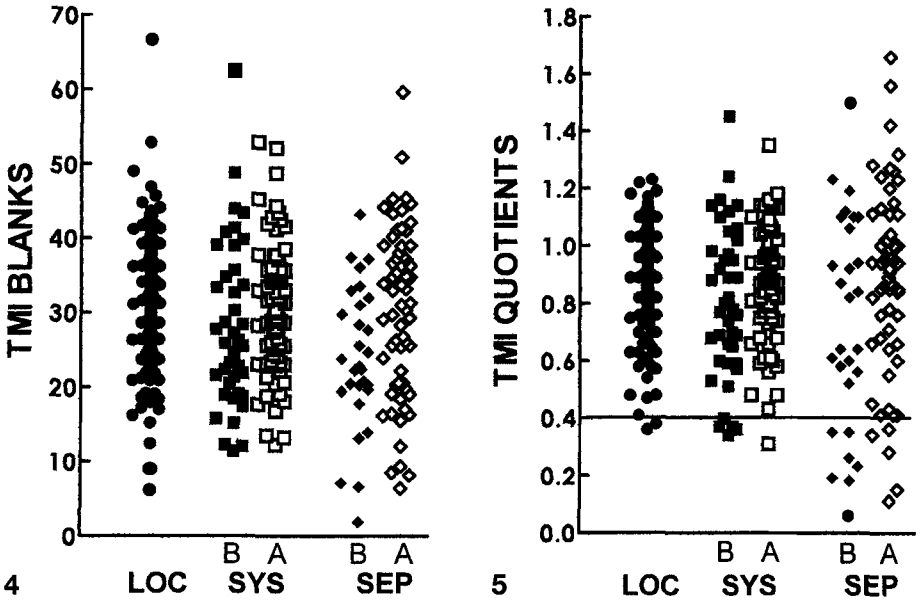

Fig. 4 PMN migration. The TMI under blank conditions. Symbols as in Fig. 1. It was not possible to establish a significant discriminating limit

Fig. 5 PMN migration. The FMLP/blank quotients of the TMI. Symbols as in Fig. 1. Values below the discrimination limit of 0.4 before onset of inflammation are considered to be predictive. The proportions between "not predictive" (above 0.4) and "predictive" (below 0.4) are for LOC 159:2, for SYS B 35:5, and for SEP B 22:7. Contingency tables: LOC-SYS**, LOC-SEP ***, SYS-SEP n.s.

Table 2 Group II, patients who developed infections with systemic involvement (SYS). Abbreviations as in Table 1, with additions: *INF* the interval in days between transplantation and the first clinical symptoms of infection, *DIFF* the interval in days between the first indication of immunodeficiency *(IDE)* and the onset of an infection *(INF)*

Pat	EL	TMI	Q	PMN	REJ	IDE	INF	DIFF	TX
24	–	–	–	–		–	7	–	LTX
25	–	–	–	+		–	14	–	HTX
26	+	–	–	–		–	13	–	LTX
27	+	–	–	–		–	11	–	LTX
28	+	–	–	–		–	5	–	LTX
29	–	–	+	–		–	17	–	KTX
30	–	+	–	–	R	17	18	1	LTX
31	+	+	–	–	R	12	20	8	KPTX
32	+	+	+	–	R	1	11	10	LTX
33	+	+	+	–	R	12	23	11	LTX
34	+	+	+	–	R	3	8	5	LTX

Table 3 Group III, patients who developed sepsis (SEP). Abbreviations as in Table 2. Patient 35 is a non-responder. The other patients showed the predictive stigmata of an impending infection – a combination of positive EL, TMI, Q and/or PMN – up to two weeks before the clinical manifestation *(DIFF)*

Pat	EL	TMI	Q	PMN	REJ	IDE	INF	DIFF	TX
35	–	–	–	–		–	6	–	LTX
36	+	+	–	+	R	5	20	15	KPTX
37	+	+	–	+		19	21	2	KPTX
38	+	+	+	–		3	18	15	LKTX
39	+	+	+	–	R	2	10	8	LTX
40	+	+	+	–		1	15	14	LTX
41	+	+	+	+	R	6	12	6	KPTX

Other parameters

As for the relative penetration depth of the migrating PMNs into a filter as represented by the DC, it was, contrary to the relative numbers of migrating PMNs as expressed by the TMI, not possible to set discriminatory limits between the three groups, either under FMLP stimulation, or in blanks or quotients. Likewise, predictive criteria could not be established for stimulated ROS-release, malondialdehyde, neopterin, sICAM-1, and sVCAM-1. Neopterin, sICAM-1 and sVCAM-1 values deviating from the collective concentrated in some subjects. Evidently, such features are individual peculiararities. High neopterin values predominantely occured after onset of infection and thus may be a reaction to the infection.

Tables 1, 2 and 3 show the individual situations and illustrate the predictive relevance of certain inflammation parameters. In group III (SEP), which includes the patients with the most severe infections, in six of seven patients (85.7%) the two predictive criteria, postsurgical elastase levels above 100 µ/l and a TMI-FMLP below "12", were combined (Table 3).

Simultaneously, PMN blood counts above 15.000 per µl and/or an FMLP to blank quotient below 0.4 were found. This configuration can predict an infection for periods between 2 and 15 days. One patient, however, is an exception and does not fit at all into the predictive pattern. When the same criteria are applied to group I (without or with local infections, LOC), only one patient was positive (4.3%). Group II (infections with systemic involvement, but not septic, SYS) lies between these two extremes. Four of the eleven patients showed the predictive combination of elastase and TMI-FMLP. All of them had a graft rejection episode. The infections

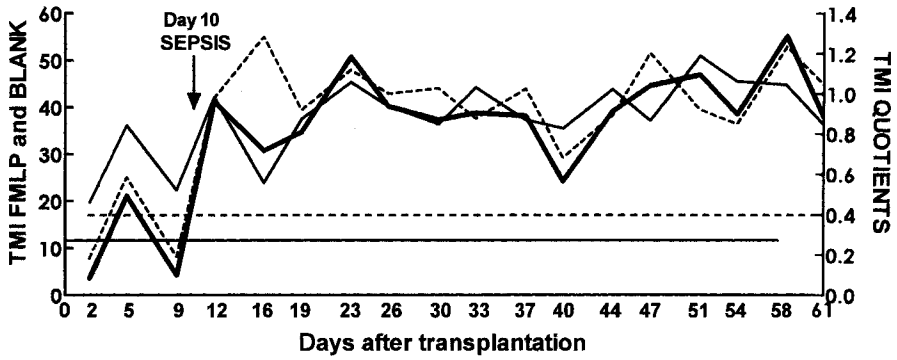

Fig. 6 The time profile of the total migration indices (TMI) of patient 39, who developed sepsis (see Table 3). *Abscissa* Days of checks after transplantation. *Left ordinate* TMI-FMLP (*full stroke*); the lower limit is 12. TMl-blank (*fine line*). *Right ordinate* The FMLP/blank quotient (*dotted line*). The lower limit is 0.4. On days two and nine, the TMI-FMLP and the quotients were below the lower limits. Sepsis occurred on day 10. During and after antibiotic treatment, the migration values normalized and were even elevated

became apparent 5–11 days after their announcement by low TMI-FMLP values. A comparison of the three groups by contingency tables yielded: LOC:SYS *, LOC:SEP*** SYS:SEP n.s.

Discussion

The rationale for this diagnostic approach of determining various PMN functions is based on the trivial experience that a human organism with an intact immune system can cope with normal microbial stress, while deficiencies in the host defence lead to infections.

Immunosuppressive therapy, unavoidable in transplantation in order to prevent graft rejection, aims at controlling the specific immune system, which makes the host defence rely on the unspecific immunity and its main representative, the PMN. Particularly in the early post-transplant phase, when the immunosuppressive therapy has to be intensive, a restriction of PMN efficiency opens the way for the main targets of PMNs – a series of bacterial and fungal pathogens – and allows them to invade and finally to attack the organism until the clinical manifestation of a reaction that is called "inflammation" becomes evident. PMN functional deficiencies bear a risk of infection, and their early diagnosis may be a means of predicting infections.

The aim of this study was to find reliable criteria to determine such PMN-functional impairment. The best predictive marker proved to be the combination of elastase and migration. Blood levels of PMN elastase above 100 µg/l during the first days after sugery, followed by a drop below 12% of circulating PMNs ready to migrate on FMLP stimulation, (the TMI-FMLP) predicted severe septic complications 2–15 days prior to their clinical manifestation with a sensitivity of 85.7% and were able to distinguish sepsis from uneventful recovery with a specificity of 95.6%. Simultaneous PMN blood counts above 15.000/µl and TMI quotients below 0.4 confirmed the value of these two parameters. Persisting low TMI-FMLP values after manifestation of an infection (Fig. 3) may be an indication for continuing ABI treatment and/or for reducing immunosuppression. Group II patients with infections with systemic involvement were found to have their markers in a medium range. Seven patients showed the functional features of the uneventful recovery of group I. The remaining four patients had positive elastase and TMI-FMLP reactions indicating infections, but the inhibition of migration was less pronounced when compared to group III (c.f the Figs. 3 and 5). Thus, the degree of inhibition might be helpful in estimating the threat of an infection.

From an upcoming study on patients treated with corticoids, where migration was measured daily, we know that the degree and duration of suppression of PMN migration are meaningful details in assessing infection risk (unpublished). In the present study, organizational reasons have forced us to perform investigations at three- and four-day intervals. This design is too wide-meshed to record all of the often desultory changes in the time profile, and much information was lost. Automation is developing, however, and will facilitate the clinical application of the migration test.

The other inflammatory parameters, relative penetration depth into a filter (the DC), ROS production, MDA, neopterin, sICAM-1, and sVCAM-1, turned out to be of no predictive value. It is noteworthy that the classical indicator for the activity of the unspecific defence system, the PMN blood count, is of no predictive significance. In no case did the number of PMNs remain under 1000 per µl, which is usually considered to be the critical lower level for a functioning immune system. In contrast, three patients in the sepsis group repeatedly had PMN numbers above 15.000/µl before the onset of inflammation (Fig. 1, Table 3). Thus, not the number of PMNs, but *functional* markers are predictive for infections. The PMN blood count, elastase release and migratory functions are independent qualities and do not correlate with each other [9,18].

Terms such as "surgical stress" and "deficiency of the defence system", though frequently used, are only poorly defined, and an attempt to analyze the pathomechanisms behind the "proneness to infections" we have found in the "immunocompromised" transplant pa-

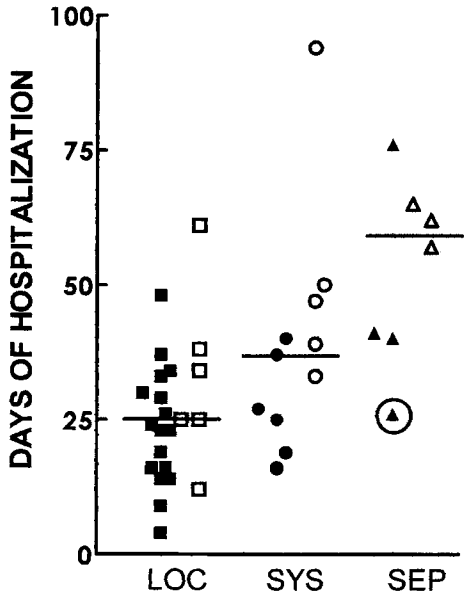

Fig. 7 Days of hospitalization of the patients with uneventful recovery *(LOC)*, with infections with systemic involvement *(SYS)* and with sepsis *(SEP)*. *Left column, full symbols* Patients without rejection. *Right column, empty symbols* Patients with rejection. The 0 patient died of sepsis. A Kruskal-Wallis ANOVA between LOC-SYS-SEP yielded a difference with $P = 0.0007$. Loc-SYS n.s., LOC-SEP***, SYS-SEP n.s. The *lines* indicate the medians The deceased patient was excluded from calculations. The length of hospitalization depended on infection, not on rejection

tients must remain fragmentary. Elastase, a product of activated PMNs [19], is generally high after surgery and decreases to normal in the recovery phase [16, 17, 22]. A strong elastase release by PMNs in the state of immunosuppression, as we have found in some of our transplant patients (we empirically set the limit at 100 μg/l), can be interpreted as a strong activation of PMNs by the surgical trauma. When high postsurgical elastase levels were accompanied or followed by TMI-FMLP values below "12 ", infections occurred in all of the 11 patients in question (the patients 31–34 of Table 2, and 36–4 1 of Table 3) except one (patient 23 of Table 1). The reactivity to blanks was much less impaired (Fig. 4), resulting in a low FMLP/blank quotient (Fig. 5, Tables 2, 3). The coincidence of high elastase release and migratory behaviour of PMNs suggests causal relationships. Agents released during traumatic stress such as cytokines may be the link between surgical trauma and an inhibition of migration. TNFα was found to inhibit the chemotactic response of blood PMNs to FMLP in vitro in a dose-dependent manner [2, 27]. Our migration assay confirmed this "negative priming". Both TNFα and IL1 added to whole blood inhibited or, depending on the dose, could also increase the response to FMLP in relation to blanks [7]. A second assault on the host defence is immunosuppressive therapy. Exogenous and endogenous immunosuppression may accumulate to impair host defence. A more scrutinious analysis of the correlations between the length of the operation, endogenous host defence, graft rejection, and the various therapies is beyond the scope of this paper and will be published elsewhere.

The source of infection is certainly of importance. The surgical wound, indwelling catheters or translocation of pathogens from the intestinal lumen into mesenteric lymph nodes are the most common ones. All septic patients underwent either LTX or KPTX. During LTX, blood flow is usually occluded for 40 to 60 min. After reperfusion this may lead to the formation of reactive oxygen species that impair the intestinal barrier, thus facilitating the invasion and the homing-in of germs, preferably in the mesenteric lymph nodes, from where they can spread into the entire organism. A comparable scenario was observed and studied during shock and polytrauma [1, 24, 26, 28] and may also be true for transplantation. Initially, the propagation of pathogens may be suppressed by the ABI prophylaxis routinely applied in the posttransplant phase. After the discontinuation of ABI, these pathogens have the opportunity to thrive and to attack the host. This mechanism could explain the sometimes amazingly long interval between the first breakdown of migration and the manifestation of an infection (Tables 2, 3).

It would certainly be ideal to prevent rather than treat infections. Apart from all the benefits for the patient, early intervention could also essentially lower the costs of transplantation (Fig. 7).

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