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Lipopolysaccharide-binding protein as a new and reliable infection marker after kidney transplantation

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Abstract The early and reliable differentiation of rejections, viral infections and bacterial infections is one of the main problems after organ transplantation. One promising solution to this problem is the lipopolysaccharide-binding protein (LBP), which is regulated upwards in gram-negative sepsis and related conditions. Therefore, the aim of our study was to explore the diagnostic potential of LBP serum levels in well-defined, non-infectious and infectious events after kidney transplantation (KTx). In a retrospective study the LBP serum levels were measured in a total of 686 serum samples from 52 kidney graft recipients. In all pre-KTx sera tested, the mean LBP level was 8.8 ± 3.5 μg/ml (reference range: 2.0–15.2 μg/ml). In 7 of 52 recipients without intraoperative T-cell depletion, the mean LBP level was significantly ($P < 0.01$) increased (13.0 ± 1.5 μg/ml) at post-KTx day 1, but was within the reference range. In contrast, the intraoperative T-cell depletion by antilymphocyte antibodies resulted in a significant ($P < 0.01$) increase to 25.8 ± 11.4 μg/ml (range: 13.3–47.2 μg/ml). In recipients with immediate ($n = 14$) or delayed ($n = 9$) graft function without any other complications, all post-KTx values (except the post-KTx peak) were within the reference range. In 10 recipients with steroid-sensitive rejections and in 11 recipients with steroid-resistant rejections, no rejection-associated changes of the LBP levels could be shown. In six recipients with cytomegalovirus infection, the detection of an antigenemia (pp65) also was not associated with alterations of the LBP levels. In addition, there was no correlation between LBP levels and the number of pp65-positive leukocytes in peripheral blood. In contrast, a strong elevation of LBP levels was seen in five recipients with gram-positive bacteremia as well as in other severe bacterial infections (e.g., purulent extravasate, heavily infected grafts, bacterial pneumonia and contaminated hematoma). In two recipients with superinfected (bacterial and mycotic or viral) *Pneumocystis carinii pneumonias* requiring assisted ventilation, LBP levels were elevated, too. Thus, in our study only systemic non-viral infections and massive lymphocytolysis were associated with elevated LBP serum levels.

Keywords Lipopolysaccharide-binding protein · Kidney transplantation · Rejection · Bacteremia · Cytomegalovirus infection

Introduction

After kidney transplantation (KTx), one of the main problems – also with respect to the therapeutic regimen required – is the early and reliable differentiation of rejections and infections (bacterial and non-bacterial). In 1986, Tobias et al. [4] described a new, acute-phase reactant in rabbit serum, the lipopolysaccharide-binding protein (LBP).

The LBP is a member of the lipid transfer/lipopolysaccharide-binding protein family that also includes the cholesteryl ester transfer protein (CETP), phospholipid transfer protein (PLTP) and bactericidal, permeability-increasing protein (BPI). These proteins share significant sequence homology, and all bind lipophilic substrates [45].

The LBP is a 58 kDa protein, mainly synthesized in the liver, which binds with high affinity to lipopolysaccharide (LPS) in plasma [42, 47]. After the binding of LPS, a LBP-LPS complex is formed. This complex is capable of transferring LPS to CD14, a glycoprotein expressed on monocytes and neutrophils. It is essential for the induction of an inflammatory response to LPS, an integral component of gram-negative bacterial cell membranes. Soluble CD14 is also able to interact with circulating LPS/LBP complexes and confers LPS responsiveness to cells (e.g., endothelial cells), which normally lack surface CD14 [35]. LPS induces an increase of LBP production in the liver within 15 to 30 min after exposure [36] with a maximum serum level after 24–48 h [43]. Once the understanding of the relationship between LPS and sepsis or related conditions grew, the diagnostic and/or prognostic values of serum LBP levels in patients with major infections were investigated. Opal et al. [34] reported the elevated LBP levels in patients with severe sepsis and/or septic shock, and Myc et al. [32] reported on patients with systemic inflammatory response syndrome. In patients with acute respiratory distress syndrome, the LBP levels also increased markedly in bronchoalveolar lavage fluid [30]. Interestingly, Froon et al. [8] measured LBP plasma levels in patients with gram-negative ($n=36$) or gram-positive (consequently lacking LPS, $n=28$) bacteremia and found no differences between both groups.

The upward regulation of LBP in bacterial infections also may be of great diagnostic importance in the field of organ transplantation. Therefore, the aim of our study was to investigate the diagnostic potential in several well-defined non-infectious and infectious events after kidney transplantation.

Material and methods

Study population

A total of 45 recipients [mean age, 43.8 ± 11.2 years; females, 16 (35.6%); males, 29 (64.4%)] who underwent cadaveric renal transplantation (first: 43, second: 1, third: 1) between April 1994 and February 1997 at the Kidney Transplant Center in Berlin-Friedrichshain were included in this retrospective study. A total of 695 serum samples (672 from kidney graft recipients and 23 from kidney graft donors) kept at –20°C were available for retrospective analysis.

In addition, seven recipients (mean age, 44.3 ± 14.8 years; females, 2; males, 5) who underwent first cadaveric renal transplantation between August 1989 and January 1990 served as a control group in order to study the influence of the surgical trauma without the concomitant intraoperative infusion of polyclonal antilymphocytic antibodies. Only the pre- and postoperative sera ($n=14$) from these recipients were analyzed.

Study design

Concentrations of LPB were measured retrospectively in serum samples from recipients with the following well-characterized postoperative courses or complications: (A) immediate post-KTx graft function without any complication within the first 3 weeks ($n=14$); (B) delayed graft function without any other complication within the first 3 weeks ($n=9$); (C) influence of surgical trauma with ($n=15$) or without ($n=7$) intraoperative antilymphocyte bolus infusion; (D) steroid-sensitive rejection episodes without signs of concomitant infections ($n=10$); (E) steroid-resistant rejections without signs of concomitant infections ($n=11$); (F) cytomegalovirus infections ($n=6$); (G) patients with bacteremia ($n=5$) or other major infections (e.g., pneumonia, purulent extravasate, heavily infected grafts).

Immunosuppression

Basic immunosuppression for all recipients in this study consisted of azathioprine (AZA), corticosteroids and cyclosporine. Details of our immunosuppressive regimen have already been published [23, 25].

A total of five recipients were switched from AZA to mycophenolate mofetil in the postoperative course at days 4, 5, 20, 280, 306 and 598, and three recipients were switched from cyclosporine to FK 506 at days 30, 32 and 38.

In addition, since February 1990 all recipients have received the Friedrichshain variant of antilymphocyte globulin induction therapy [22, 23, 25]. Briefly, this induction consisted of an intraoperative high-dose single ATG bolus in the operating theater before completion of anastomoses (i.e., the removal of clamps) via a central venous catheter. To avoid a cytokine release syndrome, 500 mg MP were given about 60 min pre-ATG. The dose used was always three times higher than recommended by the manufacturers for treatment of rejections.

Monitoring for rejection

For the diagnosis of rejection, the following clinical and laboratory signs were decisive: enlargement and tenderness of the graft, an increase in serum creatinine, concomitant change in blood urea nitrogen, oliguria, albuminuria, immunoglobulinuria, sonographic changes and immunoactivation in fine-needle aspiration cytology [21]. The treatment consisted of 5 mg/kg b.w. MP for 5 consecutive days. Polyclonal antilymphocyte globulin using a dose-by-T-cell protocol (aspired values: 50–150 T cells/μl) was used to try to reverse the biopsy-proven MP resistant rejections. The relative number of T-cells ($CD3^+$) was determined by flow cytometry (FACScan, Becton Dickinson, Heidelberg) using IQP monoclonal antibodies (IQ Products, Groningen, The Netherlands). OKT3 (2.5 mg for 10 days; CILAG, Sulzbach, Germany) was given as a rescue therapy, primarily in cases of humoral/vascular rejections. Humoral

rejection crises were proven by the detection of donor-reactive lymphocytotoxic complement dependent antibodies (DRA) using cryopreserved donor spleen cells as target cells [10].

Monitoring of cytomegalovirus (CMV) infection

All recipients and donors were screened pre-KTx for CMV-specific IgG antibodies. In the CMV-seropositive donor/CMV-seronegative recipient combination, all recipients received CMV immunoglobulins prophylactically (Cytotect, Biotest, Dreieich, Germany) at postoperative days 1 (2 ml/kg), 18 (2 ml/kg) and 35 (1 ml/kg).

Since July 1995, all recipients were screened after the transplant for CMV-pp65-antigen at least once a week using the commercially available CINAkit (Argene Biosoft, distributed by VIVA, Hürth/Cologne). Details of test performance and results have already been published [18].

Post-transplant serological diagnosis of CMV infection was carried out by the detection of CMV-specific IgM (seroconversion) and/or IgG antibodies (IMx CMV IgM and IgG, Abbott, Wiesbaden).

The treatment of CMV disease depended on the severity of the clinical symptoms and included the application of human immunoglobulins with a high content of CMV-specific antibodies (Cytotect, Biotest) and/or ganciclovir (Syntex, Aachen) as well as the cessation or dose reduction of AZA.

Monitoring of bacterial infections

Blood culture tests from patients with signs of systemic infections were performed at several different times. All blood cultures were observed for at least 7 days.

The detection of *Pneumocystis carinii* infections was carried out in bronchoalveolar lavage by means of indirect immunofluorescence.

Detection of lipopolysaccharide-binding protein (LBP) in serum

Serum samples for LBP assay came from our serum bank, containing all sera taken before transplantation and 3 times a week thereafter (always between 7:00 and 8:00 a.m.) up until discharge and also after re-hospitalization.

LBP levels were measured in one run by a commercially available assay (IMMULITE LBP; Order Code LKLB1, DPC, Los Angeles) according to the manufacturer's guidelines, using an appropriate immunoanalyzer (IMMULITE, DPC, Los Angeles [1]).

Results

Pre- and post-transplant serum LBP levels and their dependence on basic immunosuppression

Figure 1 shows the impact of the ATG bolus on the post-KTx LBP serum level. In all pre-KTx sera tested ($n = 50$), the LBP concentrations were within the reference range. The mean LBP concentration was determined to be 8.8 µg/ml. A total of 7 out of these 50 recipients received no intraoperative ATG bolus. At post-KTx day 1, the mean LBP concentration (13.0 µg/ml) was significantly increased ($P < 0.01$) but was still within the reference range. In kidney graft recipients

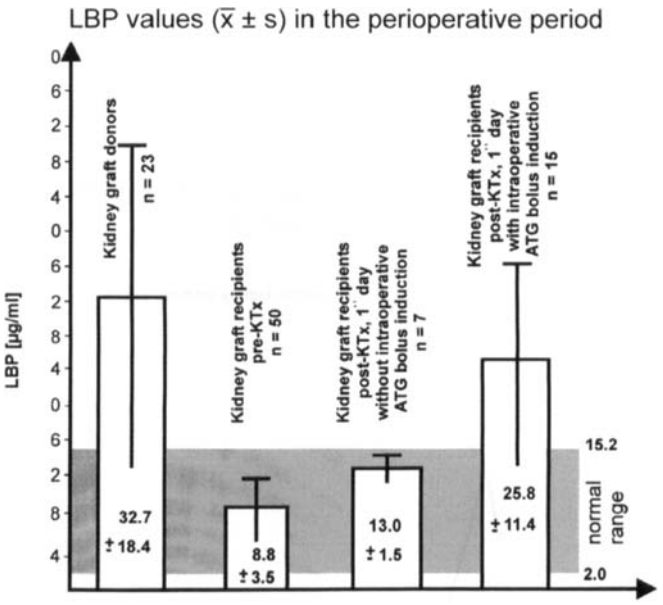


Fig. 1. LBP serum levels in kidney graft donors and recipients pre- and post-transplantation with and without intraoperative T-cell depletion

prophylactically treated with an intraoperative ATG bolus, the mean LBP serum level was determined to be 25.8 µg/ml at post-KTx day 1 (sera from 15 recipients were available for testing). This concentration was significantly higher than the pre-KTx value ($P < 0.01$) and the post-KTx level without ATG induction ($P < 0.01$) as well as the upper limit of the reference range ($P < 0.01$). Interestingly, in comparison to the pre-transplant LBP level in the recipients, the mean LBP level of the graft donors ($n = 23$) was still higher and came to 32.7 µg/ml (range: 11.9–70.7 µg/ml); only 5 out of 23 sera (21.7%) showed LBP concentrations within the reference range. However, it is worth noticing that a high donor LBP level was not necessarily associated with a worse post-KTx course. Recipients with excellent postoperative courses without any complications and immediately functioning kidney grafts received kidneys from donors with the following LBP concentrations (µg/ml): 14.1, 23.3, 29.2, 42.9 and 55.6.

Serum LBP level and its dependence on post-transplant graft function

The behavior of pre- and post-KTx LBP serum levels in kidney graft recipients with immediate or delayed graft function without any complications during the first 3 post-KTx weeks is shown in Fig. 2. Apart from a post-KTx LBP peak in both groups at days 1–2, all other pre- or post-transplant concentrations were within the reference range. There was no difference in

the LBP post-KTx dynamics between the two courses analyzed.

Serum LBP level and steroid-sensitive rejection episodes

A total of ten recipients with well-functioning grafts who experienced steroid-sensitive rejection episodes without any signs of a concomitant infection could be analyzed. The mean serum creatinine level rose in the pre-rejection week from 155.3 to 202.2 μmol/l. After a peak of 224.0 μmol/l at the beginning of therapy (5×5 mg/kg MP), the creatinine level started to decline. As shown in Fig. 3, during this 3-week-period the LBP level did not change at any time. Thus, no elevation of the LBP level was induced by a steroid-sensitive rejection.

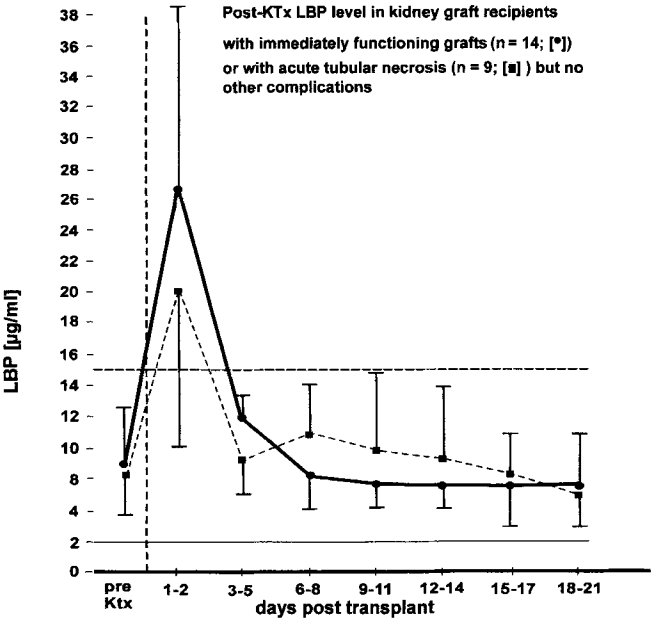


Fig. 2. Post-transplant dynamics of LBP serum levels in recipients with immediate or delayed kidney graft function

Serum LBP level and steroid-resistant rejection episodes

In 11 recipients with steroid-resistant rejections, the mean creatinine concentration rose in the pre-OKT3/ATG week from 285 μmol/l to 415 μmol/l in spite of the pre-treatment with 5×5 mg/kg b.w. MP. This was the prerequisite for biopsy and starting antibody therapy. As shown in Fig. 4, there was a slight but not significant increase in the LBP level from 10.0 μg/ml (day −2/−1 pre-OKT3/ATG) to 12.9 μg/ml (peak at day 4 to 6 during OKT3/ATG), although both mean values were within the LBP reference range.

Serum LBP level and CMV infection

The LBP serum level (mean ± 1 standard deviation) in relation to the first detection of the CMV antigen pp65 in peripheral blood polymorphonuclear cells is demonstrated in Fig. 5. In order to compare the six individual courses, the laboratory data were arranged according to the first day of pp65 detection (post-KTx days 15, 17, 22,

Fig. 3. LBP serums levels in connection with steroid-sensitive rejections

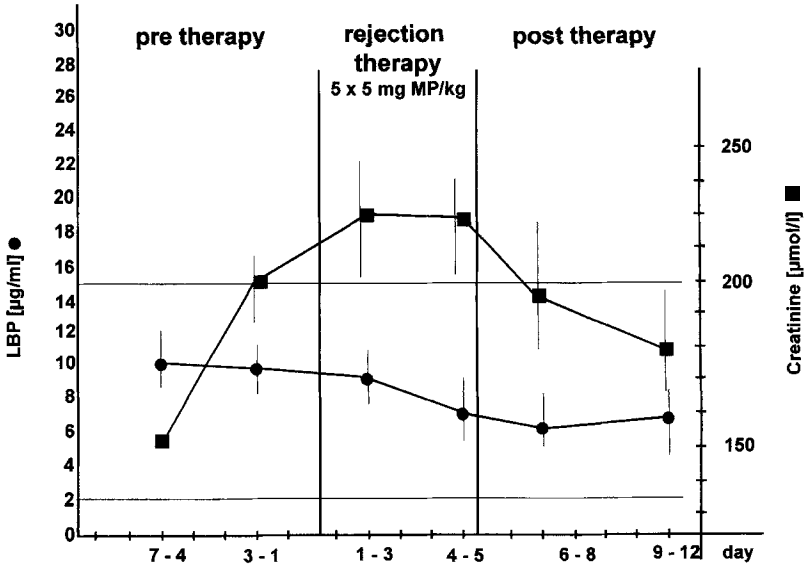
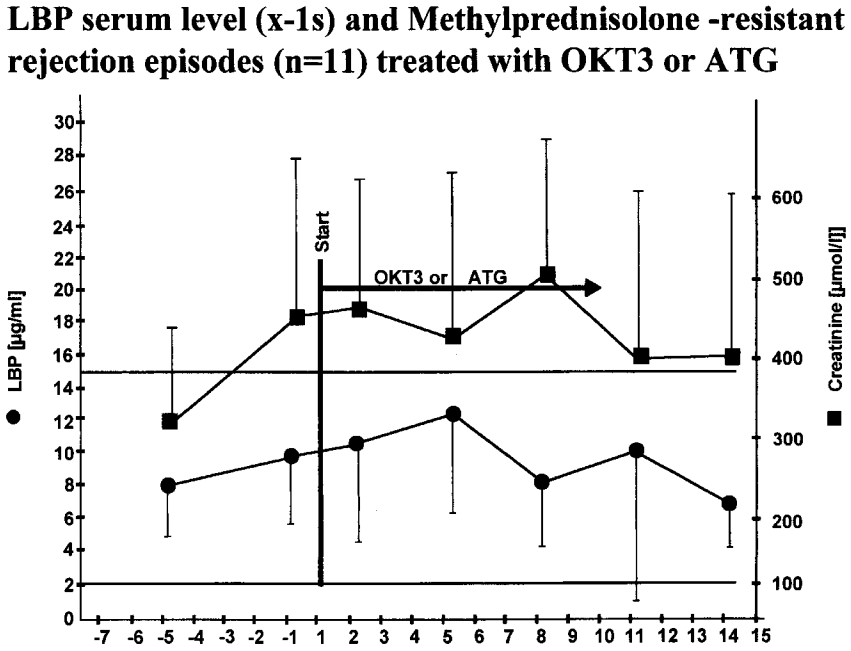


Fig. 4. LBP serum levels in connection with steroid-resistant rejections



23, 27 and 29; $x \pm s = 22.1 \pm 5.4$). In Fig. 5, the day +1 is the first day of pp65 detection in all recipients. Figure 5 shows that CMV antigenemia (without concomitant bacterial superinfection) was not associated with elevations of the LBP levels. In addition, there was also no correlation between LBP concentration (mean values in parentheses) and the number ($n = 40$) of CMV pp65 positive leukocytes/2×10^5 leukocytes (0 $pp65^+$ cells: 11.2 µg/ml LBP; 1–5 $pp65^+$ cells: 12.8 µg/ml LBP; 6–10 $pp65^+$ cells: 8.1 µg/ml LBP; 11–20 $pp65^+$ cells: 10.2 µg/ml LBP; 21–50 $pp65^+$ cells: 8.4 µg/ml LBP; > 50 $pp65^+$ cells: 7.8 µg/ml LBP).

Serum LBP level in recipients with bacteremia

Figure 6 shows a clear association between bacteremia and a strong elevation in the concentration of serum LBP. At the point of the first positive blood culture, the LBP concentrations in the five recipients studied were 18.8, 24.8, 34.1, 39.5 and 43.3 µg/ml. At this time the following bacteria could be identified: *Staphylococcus aureus* (three times), plasmacoagulase-negative staphylococcus (one time), gram-positive coccus (one time). The concomitant laboratory and clinical signs of infec-

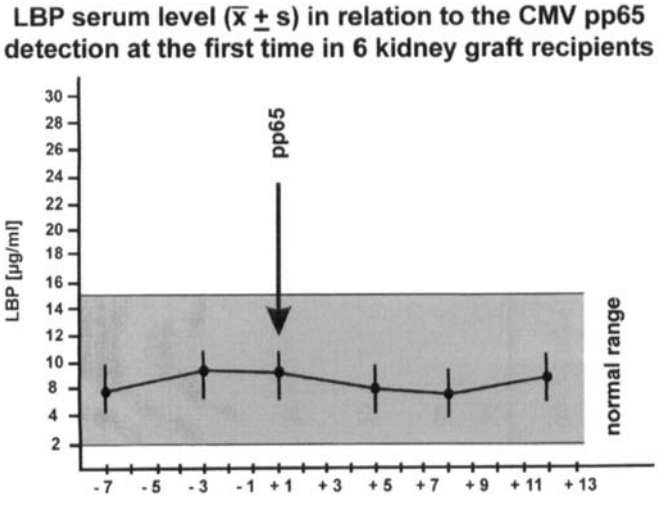


Fig. 5. LBP serum levels in kidney graft recipients with cytomegalovirus infection indicated by pp65 antigenemia

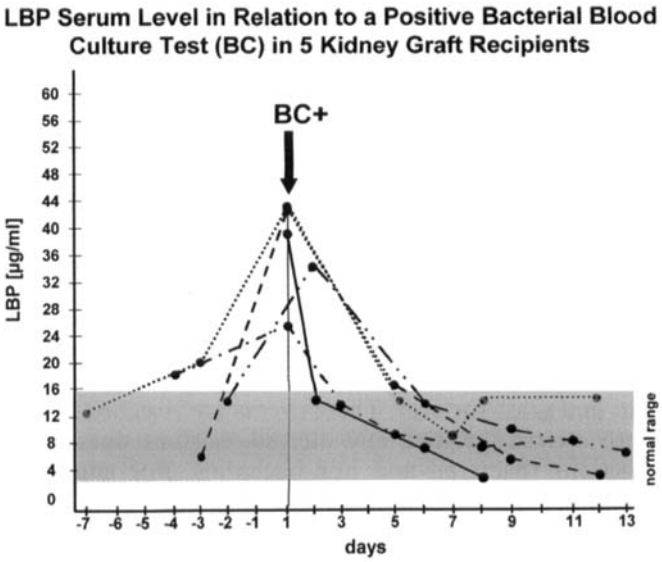


Fig. 6. LBP serum levels in five patients with bacteremia

tion and the outcome of patients are documented in Table 1. Under an efficient antibiotic regimen, the LBP serum level declined and at day 6 after the initiation of antibiotics, all LBP values were within the reference range again. Interestingly, in all cases the duration of elevated LBP levels was shorter than that of elevated CRP levels. In all five recipients the body temperature was markedly increased, but leukocytosis could be seen only in three cases.

Serum LBP level in different post-transplant complications

Serum levels of LBP that were greater than 15.2 μg/ml (the upper limit of the reference range) were detected in 123 out of 686 serum samples or in 31 out of the 52 selected kidney graft recipients. In 9 out of these 31 recipients, only one LBP value greater than 15.2 μg/ml could be found. In every case this one elevated LBP value occurred within the immediate postoperative period as a result of the intraoperative ATG bolus infusion. In 22 out of these 31 patients the number of elevated LBP values varied from 2 to 15. The association of these values with the clinical status of the recipients is shown in Table 2. All 5 of the 22 patients with bacteremia are already characterized in Table 1. The main reasons for LBP level elevations in the other 17 recipients were deep and serious wound infections accompanied by pyogenic extravasates or hematomas, bacterial_pneumonias, heavily infected grafts pre-ectomy and superinfected (bacterial and viral or mycotic) *Pneumocystis carinii* infections requiring assisted ventilation. In these patients the LBP values varied from 15.6 to 58.5 μg/ml with a mean value of 29.4 μg/ml. A more detailed analysis of these data is shown in Table 2.

Thus, the only exception for a non-infectious LBP elevation in our patients is the massive lymphocytolysis induced by a high-dose single intraoperative ATG bolus. Febrile reactions during ATG/OKT3 therapy point towards concomitant infections, because this type of anti-rejection therapy is normally not associated with elevated LBP serum concentrations (Fig. 4).

Discussion

From a clinical point of view, in the early post-transplant period there are at least two events which influence patient and graft survival. These events are rejection crises (both steroid-sensitive and steroid-resistant ones) and infections (bacterial and non-bacterial). For adequate treatment, the early and reliable diagnosis is of great importance. Both events induce inflammatory changes, which are already used within the diagnostic procedure (e.g., leukocyte count, C-reactive protein). The limitation

Table 1. Patients with positive blood cultures (BC+): a summary of the data. (LBP, CRP and body temperature from the same day). *CVC* central venous catheter, infected tips in both cases, *staph.* staphylococcus, *max./duration* maximum value/duration of elevation in days

Recipients (code)	Post-KTx day of BC+	Agent and origin	LBP (μg/ml) max./duration	CRP (mg/l)/ duration	Body temperature (°C) /duration	Leukocyte count (Gpt/l)	Last LBP value <15.2 pre-elevation	Therapy and outcome
MM-3	10	*Staph. aureus*, CVC	18.8/4 days	134.0/8 days	39.6/8 days > 38	Max. 15.0	4 days	Antibiotics, removal of CVC, cure
OI-1	44	*Staph. aureus*, pyogenic extravasate close to the graft	34.1/7 days	123.1/12 days	40.0/10 days > 38	Max. 13.7	9 days	Drainage, antibiotics, only short-time improvement
HH-2	15	*Staph. aureus*, CVC, pyogenic abscess of the abdominal wall	43.3/8 days	167.9/15 days	39.8/9 days > 38	Max. 18.9	7 days	Surgery, removal of CVC, antibiotics, cure
HW-2	51	Plasmacoagulase-negative staph., vascular rejection, OKT3 therapy	24.8/5 days	41.3/11 days	38.5/5 days > 38	Normal	7 days	Antibiotics, cure
SA-1	4	Gram-positive coccus, pneumonia	39.4/2 days	64.0/5 days	39.2/1 day > 38	Normal	4 days	Antibiotics, cure

Table 2. LBP, CRP and body temperature in infectious complications after kidney transplantation. *NA* not available

Recipients (code)	No. of elevated LBP values	LBP maximum value (μg/ml)	CRP same day (mg/l)	Body temperature, same day (°C)	Duration of LBP elevation (days)	Clinical picture/status
SD-1	2	30.2	52.5	37.6	3	Both values immediately post-transplantation
MT-2	2	18.8	173.0	37.2	6	Deep wound infection, *Staphylococcus aureus*
MM-3	2	18.8	134.7	39.5	4	See Table 1, *Staphylococcus aureus*
BJ-4	2	27.7	144.0	38.6	3	Post-graftectomy sepsis
SA-1	3	39.5	64.0	39.2	2	See Table 1, gram-positive coccus
SK-2	3	18.5	25.9	37.8	4	Pyogenic extravasate
LA-3	3	23.4	51.0	38.1	6	Superinfected CMV disease
TU-1	4	22.8	24.8	38.0	3	Pneumonia, rejection
HH-2	4	43.3	167.9	39.8	8	See Table 1, *Staphylococcus aureus*
GW-3	4	23.8	33.9	38.8	7	Pneumonia, graftectomy
AU-4	4	24.5	97.0	38.2	11	Pneumonia, pyogenic wound infection
BH-5	4	29.3	118.0	38.0	6	Purulent kidney cysts, nephrectomy
SK-6	4	53.7	354.1	NA	>5	Mycotic superinfected *Pneumocystis carinii pneumonia*
SG-1	5	32.9	63.0	39.4	NA	Purulent kidney cysts
HW-2	5	24.8	43.1	37.2	5	See Table 1, staphylococcus, plasmacoagulase-negative
SE-1	6	22.3	55.2	37.8	3	Pyogenic extravasate, wound infection, vascular rejection
KH-2	6	25.1	47.9	36.6	11	Pyogenic hematoma, bleeding, surgical revision
HH-1	7	35.5	25.9	38.0	18	Fever, serious vascular rejection
WR-2	7	58.5	171.0	39.7	10	Pyogenic hematoma, *E. coli* infection of the urinary tract
BW-1	11	33.1	201.7	39.9	36	Septic-thrombotic graft, graftectomy, pyogenic hematoma
SW-2	11	58.0	134.8	NA	28	CMV superinfected *Pneumocystis carinii* pneumonia
OI-1	15	34.1	123.1	40.0	5	See Table 1, *Staphylococcus aureus*

of these parameters is their lack of specificity regarding discrimination of the underlying causes of inflammation. During the last years some new parameters have been introduced (e.g., procalcitonin [3, 4, 7, 11, 13, 33], interleukins [12, 24, 29, 31, 38], neopterin [2, 16, 44] and sCD14 [5, 28, 39]), which should allow a better classification of infections. Especially for the diagnosis and monitoring of sepsis, a novel acute-phase protein, the lipopolysaccharide-binding protein, was recently introduced [41], and in a few papers, a strong association between elevated LBP serum or plasma levels and bacterial infections has been reported [8, 30, 32, 34]. In addition, elevated LBP levels were also measured in amniotic fluid [37], synovial fluid [15], bronchoalveolar lavage fluid [30] and cerebrospinal fluid [14].

Up until now, no results on the diagnostic potential of LBP in organ graft recipients have been reported. Therefore, in a retrospective analysis the LBP serum levels were measured in a total of 709 serum samples from 52 kidney graft recipients and 23 kidney graft donors.

In the 23 kidney graft donors (23 serum samples, respectively) the mean LBP level was significantly higher than the LBP level in normal humans, indicating an acute phase reaction in the organ donor. Only 5 out of these 23 sera showed LBP levels within the reference range. Interestingly, the donor LBP level does not predict the post-transplant course of the grafted kidney; also, increased LBP levels were associated with immediately functioning grafts without any sign of infectious or non-infectious complications.

In contrast to the donors, in all of the sera from pre-transplant recipients that were tested, the LBP levels were within the normal range. These data reflect the good condition of the prospective recipients.

The first acute phase with which the recipients are confronted is the surgical trauma of grafting.

In 7 of 52 recipients without intraoperative T-cell depleting measurements at post-KTx day 1, the mean LBP level was within the reference range but significantly elevated compared to the pre-KTx level of these seven recipients (13 versus 7.3 μg/ml). This means that

the surgical trauma resulted in a significant increase in the LBP level, but the extent of the increase is without diagnostic value. In contrast to the LBP level, already in 1984 [9] we could show that in the first postoperative week the mean CRP values were over the upper limit of the normal range.

The inauguration of a new induction therapy [17, 22] consisting of an intraoperative T-cell depletion by means of polyclonal antilymphocyte globulins added a massive lymphocytolysis to the surgical trauma. This additional stress led to an increase in the mean LBP level (range: 13.3–47.2 μg/ml) at post-KTx day 1. The differences to the pre-KTx value as well as to the post-KTx value without antilymphocyte globulin induction were significant. Thus, a dramatic reduction of T-cells by means of anti-T-cell antibodies gave rise to a (non-infectious) LBP elevation. As shown earlier for ATG-Fresenius, already at the time of de-clamping (opening of anastomosis), there was a strong elevation in particular of IL-6 (interleukin-6), TNF-alpha (tumor necrosis factor alpha) [23] and IL-10 [27] immediately after finishing the ATG infusion. This intraoperative interleukin peak was followed by a CRP peak at post-KTx days 1–2 [40].

Aside from the post-KTx peak at day 1 or 2, all uncomplicated courses were characterized by LBP values within the reference limit. There were no differences between immediate or delayed post-KTx graft function.

Interestingly, in recipients who were experiencing steroid-sensitive rejection episodes, which were indicated by increased serum creatinine concentrations and other signs, and were responding very well to MP bolus therapy, there were no cases of accompanying changes in the LBP levels. This fact is very important, because we were able to demonstrate a significant increase of CRP [19, 20] prior to rejection already in 1981. Elevations of IL-6 concentrations from 13 ± 6 pg/ml to 34 ± 12 pg/ml in the week before initiating anti-rejection therapy with high doses of MP were published some years later [26], indicating the systemic reactions to the local inflammatory process within the graft. Also, in steroid-resistant rejections ensured by biopsy and characterized by a strong serum creatinine increase, there was no obvious alteration of the LBP level. After starting anti-rejection therapy with mono- or polyclonal antilymphocyte antibodies in a "normal" (in contrast to the intraoperative bolus) dosage (2.5 mg OKT3/d or 3 mg/kg b.w. ATG Fresenius/d), there was only a slight but not significant increase in the mean LBP level within the reference range. Thus, it should be pointed out that both steroid-sensitive and steroid-resistant rejection crises are not associated with increasing serum LBP levels that are above the upper limit of the reference range. Interestingly, in the rat small bowel transplantation model, Cicalese et al. [6] described the expression of CD14 and LBP mRNA as a useful tool for early diagnosis of acute rejection, but the expression was also increased in the syngeneic grafts, indicating that bacteria could also play a role.

A common infectious agent after KTx is the cytomegalovirus. This virus is regarded as a major cause of morbidity and mortality in immunosuppressed organ recipients. Both can be prevented in part by early antiviral treatment. Aside from prophylactic measurements, the early detection of CMV antigenemia by polymerase chain reaction or immunofluorescence is a prerequisite for an early therapeutic intervention (e.g., preemptive therapy). By means of indirect immunofluorescence, we correlated both the first detection of pp65 antigen as well as the number of pp65-positive leukocytes in the peripheral blood and, at the same time, the LBP level and could not detect any correlation. Thus, CMV infections without any other complications are not associated with increased LBP serum levels.

On the other hand, in all five cases studied the detection of a bacteremia was associated with a strong elevation of serum LBP. Interestingly, the five bacteria grown in the blood culture were gram-positive. Under an efficient regimen of antibiotics, the LBP levels declined, and 6 days after the initiation of antibiotics, all LBP values were within the reference range again.

The analysis of all of the data available clearly shows that the majority of elevated LBP levels occurred in relationship with bacterial infections. In two cases, strong LBP elevations were found in serious *Pneumocystis carinii pneumonias* with bacterial and mycotic or viral superinfection, which required assisted ventilation. Unfortunately, because of a lack of serum samples, we are not able to say anything about LBP serum levels in patients with systemic mycosis.

The only exception to a non-bacterial LBP elevation was the drastic intraoperative lymphocytolysis induced by an polyclonal antilymphocyte globulin bolus. The infusion of lower dosages of mono- or polyclonal antilymphocyte globulins to overcome steroid-resistant rejections were normally not associated with elevated LBP levels. In our view, all elevated LBP levels in connection with OKT3/ATG therapies point to concomitant infections.

Thus, in our study population only bacterial infections, *Pneumocystis carinii pneumonias* with bacterial and mycotic or viral superinfection (in two cases) and massive lymphocytolysis by an intraoperative ATG bolus, induced elevated LBP serum levels. The differentiation of rejections, CMV infections and bacterial infections by means of LBP seems to be possible. The determination of LBP serum levels after KTx is recommended, but it is absolutely essential to determine the correlation of the LBP results with the results of other assays (e.g., CRP, procalcitonin, interleukins, neopterin and sCD14).

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