

# The Role of Hyperleptinaemia and Low Values of Interleukin 10 in De Novo DSA Production After Kidney Transplantation

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## Research Article

**Keywords:** leptin, IL-10, protocol biopsy, kidney transplantation

**Posted Date:** December 15th, 2021

**DOI:** <https://doi.org/10.21203/rs.3.rs-762251/v1>

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**Version of Record:** A version of this preprint was published at Transplant Immunology on April 1st, 2024.

See the published version at <https://doi.org/10.1016/j.trim.2023.101982>.

# Abstract

## Background

White adipose tissue secretes a number of peptide hormones. The aim of this paper was to determine the role of leptin, adiponectin and interleukin-10 and interleukin-6 on the development of graft rejection in protocol biopsy after kidney transplantation.

## Methods

In a prospective analysis (n = 104), we monitored the values of leptin, adiponectin, IL-6, and IL-10 prior to the transplantation and in the 3rd month after the transplantation. The protocol biopsy of the graft was performed in the 3rd month after the transplantation. The group was divided into the following according to the biopsy result: negative result, IFTA 1, borderline, and DSA positive.

## Results

After adjusting for the differences in the baseline recipient and donor characteristics, we identified the hyperleptinaemia baseline (HR = 2.0444, P = 0.0341) and month 3 (HR = 49.8043, P < 0.0001) as independent risk factors for borderline changes in the protocol biopsy. The hyperleptinaemia baseline (HR = 7.4979, P = 0.0071) and month 3 (HR = 9.7432, P = 0.0057) are independent risk factors for de novo DSA positivity. A low value of IL-10 month 3 is a risk factor for de novo DSA positivity (HR = 3.0746, P = 0.0388).

## Conclusions

Higher leptin levels and low values of IL-10 might play a role in rejection and de novo DSA production.

## Introduction

It is now widely accepted that white adipose tissue secretes a number of peptide hormones, including leptin, adiponectin, resistin, and several cytokines (1). Leptin is secreted mainly by white adipose tissue, and leptin levels are positively correlated with the amount of body fat (2). The most significant roles of leptin include the regulation of energy homeostasis, neuroendocrine function, and metabolism. The other effects of leptin involve the regulation of immune function and bone metabolism (3). In innate immunity, leptin modulates the activity and function of neutrophils by increasing chemotaxis and secretion. The stimulation of human monocytes by leptin induces the production of tumour necrosis factor (TNF)- $\alpha$  and interleukin (IL)-6 (4). Leptin enhances phagocytosis by macrophages, the secretion of pro-inflammatory mediators of the acute-phase response, and the expression of adhesion molecules. In natural killer cells, leptin increases the cytotoxic ability and secretion of perforin and IL-2. In adaptive immunity, leptin

promotes the generation, maturation, and survival of thymic T cells. In naive T cells, leptin increases proliferation and IL-2 secretion. In memory T cells, leptin triggers Th1 polarisation by increasing interferon (IFN)- $\gamma$  and TNF- $\alpha$  secretion. Leptin has anti-apoptotic effects on mature T cells and haematopoietic precursors (5).

Adiponectin is a fat-derived hormone that appears to play a crucial role in the protection against insulin resistance/diabetes and atherosclerosis. Decreased adiponectin levels are thought to play a central role in the development of type 2 diabetes, obesity, and cardiovascular disease in humans. Adiponectin levels are inversely correlated with visceral obesity and insulin resistance, and weight loss is a potent inducer of adiponectin synthesis. TNF suppresses adiponectin secretion in adipocytes, and its production is also regulated by other proinflammatory cytokines, such as IL-6 (6).

IL-10 is a potent anti-inflammatory cytokine that plays a crucial, and often essential, role in preventing inflammatory and autoimmune pathologies. IL-10 is a cytokine that has structural and functional similarities to leptin (7). Few studies have addressed the influence of adipose-tissue-derived products, such as leptin, on the allograft response (8, 9). Leptin is cleared principally by the kidney. Not surprisingly, serum leptin appears to increase concurrently with declines in the glomerular filtration rate (10), and therefore, it is clear that leptin values increase in the case of clinically expressed graft rejection. However, we have asked ourselves how selected adipocytokines (leptin and adiponectin) will react in the environment of subclinical graft rejection, which is confirmed by protocol graft biopsy in the 3rd month after kidney transplantation. The role of IL-10 in graft rejection is still unclear. It is, however, known that leptin decreases IL-10 production (11).

## Materials And Methods

This study was a prospective one-centre analysis that included patients (Caucasians) older than 18 years that had undergone primary transplantation of the kidney in the Martin (2018-2019), Slovakia Transplantation Center. Patients with diabetes mellitus diagnosed prior to the kidney transplantation, patients with complicated or protracted infection, including secondary wound healing, and patients with a medical history of oncology illness were excluded from the monitoring (Figure 1). Patients who did not complete the 3-month follow-up and patients who did not undergo protocol biopsy or whose protocol biopsy result was not representative were also excluded from the monitoring. One hundred four patients who met the criteria were thus included in the study.

### Immunosuppression and induction

All patients included in the monitored group received induction according to the protocol of the Martin Transplantation Center using the anti-thymocyte globulin in a total dosage of 3.5 mg/kg (divided into three doses: day 0, day 1, day 2). The criteria for induction with the anti-thymocyte globulin are: panel reactive antibodies more than 10%, history of positive cross match test, dialysis program more than 5 years, cold ischemia time more than 18 hours or expanded criteria donor. The maintenance immunosuppression consisted of tacrolimus and mycophenolic acid (1080 mg daily dose until the 2nd

week after the transplantation, followed by a 720 mg daily dose). Methylprednisolone 500 mg i.v. was administered on day 0 and day 1, followed by the administration of Prednisone 20 mg until the 2nd week after the transplantation, Prednisone 15 mg until the 4th week after the transplantation, Prednisone 10 mg until the 12th week after the transplantation, and Prednisone 7.5 mg until the 12th month after the transplantation, with Prednisone 5 mg administered daily thereafter

### **Adipocytokines and interleukins**

We determined the leptin, adiponectin, IL-6, and IL-10 values of each patient prior to the transplantation (2 hours before the surgery) and in the 3rd month after the transplantation. The Human Total Adiponectin ELISA Kit, Human Leptin Quantikine ELISA kit, LEGEND MAX Human IL-6 ELISA Kit, and the LEGEND MAX Human IL-10 Kit were used for examination. Hyperleptinaemia was determined when the leptin value was higher than 77 µg/ml. Hypoadiponectinaemia was determined when the leptin value was lower than 9 µg/ml (based on reference values determined by the manufacturer of the kits used). Hyper-IL-6 was determined when the IL-6 value was higher than 6.4 pg/ml, and hypo-IL-10 was determined when the IL-10 value was lower than 2.8 pg/ml.

### **Other monitored parameters**

We determined the average tacrolimus (TAC) level for 3 months for all patients. We also recorded the estimated glomerular filtration rate (eGFR) (according to the CKD-EPI formula) at the time of sampling adipocytokines and interleukins. We monitored the body mass index (BMI), waist circumference (baseline, and month 3 [M3]), and lipid metabolism parameters of all patients (Table 1).

### **Protocol biopsy and monitoring of donor-specific antibodies (DSA)**

Protocol graft biopsy and the determination of donor-specific antibodies was performed in the 3rd month for all patients included in the study by means of the LUMINEX methodology (positivity was stipulated at  $\geq 500$  MFI). We further divided the group according to the result of the protocol biopsy (according to the Banff classification 2019) into groups with a negative result, interstitial fibrosis and tubular atrophy (IFTA), borderline changes (t1-t3 with i1 or t1 with i2 or i3), and group with positive donor specific antibodies. We did not observe changes in the character of T-cell-mediated rejection (TCMR) and antibody-mediated rejection (ABMR) in the monitored group. The IFTA group included only patients meeting criteria for IFTA 1).

### **Statistical analysis**

We used a certified statistical program, MedCalc version 13.1.2. (VAT registration no. BE 0809 344 640, Member of [International Association of Statistical Computing](#), Ostend, Belgium), to perform statistical analyses. Continuous data were compared using the Student's t-test or the Wilcoxon rank-sum test as appropriate. The  $\chi^2$  test and Fisher's exact test were used for categorical variables. The Cox proportional hazards model was used to adjust for the differences in baseline recipient (such as age, gender, HLA mismatch, panel reactive antibodies - PRA, and time in dialysis program) and donor characteristics (cold

ischemia time - CIT, expanded criteria donor - ECD) on the endpoint of rejection. ROC curve analysis was used for leptinaemia 3M and rejection and IL-10 and rejection. We considered a P-value of <0.05 to be statistically significant.

### Ethical approval

All procedures involving human participants have been approved according to the ethical standards of the institutional research committee, including the 1964 Helsinki Declaration and its later amendments of comparable ethical standards. Informed consent for included participants was checked and approved by University hospital's and Jessenius Faculty of Medicine's ethical committees and all signed informed consents have been archived for at least 20 years after research was completed.

The clinical and research activities being reported are consistent with the Principles of the Declaration of Istanbul as outlined in the Declaration of Istanbul on Organ Trafficking and Transplant Tourism.

## Results

One hundred four patients (men=63.5%) were included in the group. We observed a stable value of graft function (without significant differences) during the monitored period.

The leptin value did not significantly change in the group during the monitored period; however, numerically there was an increase compared to the baseline. In the case of adiponectin, we observed a significant decrease. Similarly, there was a significant increase in IL-10.

Basic characteristics of the group and subgroups according to the results of the protocol graft biopsy are shown in Table 2.

We did not observe any difference in the graft function in individual groups; there were no significant differences in BMI, CIT, time in the dialysis program, PRA, or mismatch. Between the monitored groups, there were no significant differences in the average TAC level during the first three months after the transplantation. Patients DSA+ showed the highest leptin value and the lowest IL-10 value at the time of transplantation (baseline). The leptin level in M3 was also the highest in patients with DSA+. Patients with borderline had the highest values of IL-6 in M3. This group of patients, together with patients with DSA+, also had the lowest values of IL-10 in M3.

Tables 3 and 4 show the hazard ratio for the endpoint of borderline and DSA+ after adjusting for differences in baseline recipient and donor characteristics.

We added the ROC curve analysis for leptin 3M and borderline plus de novo DSA (optimal criterion = leptin level > 39 µg/ml [specificity, 90.91%; sensitivity, 63.16%]) and for IL-10 3M and borderline plus de novo DSA (optimal criterion = IL-10 level ≤ 2.58 pg/ml [specificity, 85.71%; sensitivity, 88.24%]) (Figure 2).

## Discussion

It is well known that leptin promotes the secretion of inflammatory cytokines and the activation of macrophages, neutrophils, and natural killer cells. Functions in adaptive immunity include thymic homeostasis, naïve CD4<sup>+</sup> cell proliferation, the promotion of T helper 1 (TH1) responses, and the suppression of CD4<sup>+</sup>CD25 regulatory T cells (12). Consequently, leptin can contribute to the onset and progression of several T-cell-controlled autoimmune diseases, but its role in the development of graft rejection has not been described.

Some studies have suggested that uraemia may be associated with higher leptin levels, while others have suggested that only nutritional parameters modulate these hormones (13–15). Fonseca et al. found that decreases in leptin levels pre- and post-transplantation were associated with less delayed graft function since higher levels may increase the risk of graft loss (16). In our previous analysis published in 2019, we found that despite the increasing value of eGFR during the first 6 months after transplantation the value of leptin increased (17). We reached similar results in this analysis. The leptin levels were the lowest before kidney transplantation when eGFR was the lowest. We associate the aforesaid with the activation of leptin immune functions, but the metabolic effects of leptin can of course also play a role. However, we did not confirm any significant change in BMI or waist circumference in the group during the monitored period. Our hypothesis was also supported by the fact that patients who showed DSA<sup>+</sup> had the highest leptin values, both at the time of baseline and at the time of protocol biopsy, i.e., in M3. The assumption that the leptin value would be the highest in clinically expressed ABMR is obvious, but our analysis proves that hyperleptinaemia is also present in cases of de novo DSA positivity. Leptin values were highest in M3 in patients with de novo DSA positivity and in patients with borderline changes. The available literature has not yet defined a clear link between graft rejection and the leptin value. In 2013, [Moraes-Vieira](#) et al. described on animal model the possible effect of leptin on the allogeneic skin transplant model, where a leptin deficiency resulted in an increased frequency of Treg and Th2 cells and prolonged graft survival (18). However, clinical data are currently not available. In our group, hyperleptinaemia baseline and hyperleptinaemia in the 3rd month were independent risk factors for DSA<sup>+</sup> and borderline histological findings in the protocol biopsy..

IL-10 is known to play a critical immunoregulatory role during immune responses to microbial pathogens (19). However, the role of IL-10 in the development of graft rejection is still unclear. It is assumed that a combination of [transforming growth factor](#) (TGF)- $\beta$  and IL-10, but not single IL-10, is required to suppress the B cell activation induced by toll-like receptor stimulation. In in vivo analyses, the simultaneous presence of TGF- $\beta$  and IL-10 effectively suppressed TLR-mediated antigen-specific immune responses (20). In our group, we identified hypo-IL-10 as an independent risk factor for DSA<sup>+</sup> in the 3rd month after transplantation. Several authors have monitored gene polymorphisms of cytokines, but without a clearly proven link between the IL-10 polymorphism and the development of acute graft rejection (21, 22).

Adiponectin is considered to be an anti-inflammatory cytokine, and it also plays a key role in metabolic syndrome. Wolf et al. proved that adiponectin induces the production of anti-inflammatory mediators,

particularly IL-10. Adiponectin values decrease in the hyperleptinaemia environment, which results in a decrease in IL-10 (23). We did not confirm the role of adiponectin in relation to the rejection in our group; although, patients with borderline had significantly lower values of adiponectin in M3. IL-6 values increased slightly during the monitored period until the 3rd month after the transplantation and subsequently decreased, which we linked with the postoperative period, postoperative stress, and the release of proinflammatory cytokines in this period.

The limitation of our study was the small group of patients in the individual subgroups, according to the histological result. On the other hand, this study is one of the few studies that has monitored the development of leptin and adiponectin during the first year after kidney transplantation and the only study to describe the role of leptin, adiponectin, IL-6, and IL-10 in relation to the histological finding in the protocol biopsy performed in the 3rd month after the kidney transplantation.

## Conclusion

Adipocytokines can play an important role in the development of graft rejection of patients after kidney transplantation. Higher leptin levels may play a role in DSA production. The role of IL-10 in graft rejection is still unclear, our analysis confirmed that low IL-10 values are an independent risk factor for the development of de novo DSA production in 3<sup>rd</sup> month after kidney transplantation. We assume that values of adipocytokines in the context of other risk factors can predict the immunological risk of patients after kidney transplantation, however other studies with higher number of patients are needed for confirmation of our hypothesis.

## Abbreviations

ABMR, antibody-mediated rejection

BMI, body mass index

CKD EPI, Chronic Kidney Disease Epidemiology Collaboration

CIT, cold ischaemia time

D, day

DGF, delayed graft function

DSA, donor-specific antibody

ECD, expanded criteria donor

eGFR, estimated glomerular filtration

HLA, human leukocyte antigen

HOMA-IR, homeostatic model assessment for insulin resistance

IFN, interferon

IL, interleukin

IRI, immunoreactive insulin

IFTA, interstitial fibrosis and tubular atrophy

M, month

MFI, mean fluorescence intensity

PRA, panel-reactive antibodies

PTDM, posttransplant diabetes mellitus

TAC, tacrolimus

TCMR, T-cell-mediated rejection

TGF, transforming growth factor

TNF, tumour necrosis factor

## Declarations

### **Ethical approval and consent to participate:**

All procedures involving human participants have been approved according to the ethical standards of the institutional research committee, including the 1964 Helsinki Declaration and its later amendments of comparable ethical standards.

The clinical and research activities being reported are consistent with the Principles of the Declaration of Istanbul as outlined in the Declaration of Istanbul on Organ Trafficking and Transplant Tourism. No organs were obtained from prisoners and organs were obtained via National – Slovak transplant organization.

Informed consent was obtained from all individual participants included in the study.

**Consent for publication:** Patients signed informed consent regarding publishing their data.

**Availability of data and material:**

Informed consent for included participants was checked and approved by University hospital's ethical committee and all signed informed consents have been archived for at least 20 years after research was completed.

**Competing interests:** The authors declare no conflicts of interest.

**Funding:** The project was supported by VEGA grant 1/0023/19.

**Authors' contributions:**

Assoc. Prof. Ivana Dedinská, MD, PhD: participated in the writing of the paper, the performance of the research, and the data analysis

Assoc. Prof. Daniela Kantárová, MD, PhD: participated in the data analysis

Katarína Macháleková, MD, PhD: participated in the data analysis

Karol Graňák, MD: participated in the research design and data analysis

Matej Vnučák, MD, PhD: participated in the research design and data analysis

Prof. Ľudovít Laca, MD, PhD: participated in the writing of the paper

Prof. Marián Mokáň, MD, DrSc., FRCP Edin: participated in the research design

**Acknowledgements:** none

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## Tables

**Table 1** Basic group characteristics.

n = 104	Baseline	M3	P
Men (%)	63.5	–	–
Age at Tx (years)	46.5 ± 13.1	–	–
BMI (kg/m <sup>2</sup> )	26 ± 4	25.8 ± 4.2	0.7255
Waist circumference (cm)	–	94.4 ± 12.4	–
ECD (%)	25	–	–
CIT (min)	829 ± 388	–	–
DGF (%)	11.3	–	–
Time in dialysis program (months)	27 ± 15	–	–
PRA (%)	3.5 ± 0.5	–	–
Mismatch A	1.3 ± 0.7	–	–
Mismatch B	1.4 ± 0.6	–	–
Mismatch DR	1.3 ± 0.6	–	–
Mismatch DQ	1.1 ± 0.8	–	–
Leptin (µg/ml)	31.3 ± 22	36.4 ± 22.2	0.0976
Adiponectin (µg/ml)	19.3 ± 9.6	16.3 ± 9	<b>0.0210</b>
IL-6 (pg/ml)	25 ± 19.8	29.2 ± 22.1	0.1504
IL-10 (pg/ml)	4.9 ± 2.7	7.8 ± 4.8	<b>&lt;0.0001</b>
Fasting blood glucose (mmol/l)	–	5.7 ± 1.6	–
PTDM (%)	–	23.1	–
HbA1c (%)	–	3.7 ± 0.9	–
C-peptide (µg/l)	–	4.2 ± 2	–
IRI (mU/l)	–	8.1 ± 3.6	–
HOMA-IR	–	2.1 ± 0.5	–
TAC level (ng/ml)	–	8.8 ± 3.2	–
eGFR CKD-EPI (ml/min)	–	55 ± 22.6	–

Tx, transplantation; BMI, body mass index; ECD, expanded criteria donor; CIT, cold ischaemia time; DGF, delayed graft function; PRA, panel-reactive antibodies; IL, interleukin; IRI - immunoreactive insulin; PTDM,

posttransplantation diabetes mellitus; HbA1c, glycated haemoglobin; HOMA-IR, homeostatic model assessment for insulin resistance; TAC, tacrolimus; eGFR CKD EPI, estimated glomerular filtration by Chronic Kidney Disease Epidemiology Collaboration.

**Table 2.** Characteristics of the group according to the protocol biopsy result.

	negative n = 50	IFTA 1 n = 31	borderline n = 13	DSA+ n = 10	<i>P</i>
Men (%)	62.5	74.2	82.3	10	<b>0.0113</b>
Age at Tx (years)	45.4 ± 14.4	41.2 ± 10.1	40 ± 10.7	48 ± 10.1	0.6202
BMI (kg/m <sup>2</sup> )	27.9 ± 4.7	23.9 ± 2.5	23.6 ± 2.3	30 ± 4	0.7492
Waist circumference (cm)	98.1 ± 14	93.7 ± 11.5	87.4 ± 14.4	94 ± 9.6	0.4666
ECD (%)	25	29	15.4	20	0.4609
CIT (min)	883 ± 361	853 ± 449	474.5 ± 129	750 ± 280	0.1739
DGF (%)	7.5	9.6	7.8	10	0.2646
Time in dialysis program (months)	17.5 ± 11	24.1 ± 14	11.3 ± 6.7	21.4 ± 13	0.2017
PRA (%)	1 ± 0.7	0.7 ± 0.3	0.7 ± 0.5	1 ± 0.6	0.9017
Mismatch	1.3 ± 0.6	1.3 ± 0.7	1.7 ± 0.6	1.5 ± 0.6	0.4348
DSA (MFI)	–	–	–	5100 ± 2890	–
Leptin baseline (µg/ml)	32.6 ± 25.3	32.3 ± 23.1	41.2 ± 39.6	66.1 ± 33.3	<b>0.0161</b>
Adiponectin baseline (µg/ml)	19.7 ± 8.6	18.2 ± 6.9	21.8 ± 8.5	17.4 ± 10.2	0.0893
IL-6 baseline (pg/ml)	9.9 ± 1.1	35.3 ± 11.8	27.4 ± 16.5	37.3 ± 10.2	<b>0.0352</b>
IL-10 baseline (pg/ml)	10.4 ± 5.7	4.7 ± 2.7	5.4 ± 4.1	2.9 ± 1.4	<b>0.0236</b>
Leptin M3 (µg/ml)	39.8 ± 30.5	15.3 ± 6	47.9 ± 37.3	90.8 ± 33.8	<b>0.0008</b>
Adiponectin M3 (µg/ml)	14.4 ± 8.3	20 ± 8.4	11.3 ± 8.7	18.2 ± 6.9	<b>0.0258</b>
IL-6 M3 (pg/ml)	36.1 ± 28.2	24.5 ± 19.1	66.1 ± 21.2	35.3 ± 10.2	<b>0.0065</b>
IL-10 M3 (pg/ml)	9.7 ± 0.7	12.8 ± 9.3	3.1 ± 2.2	2.9 ± 1.4	<b>0.0057</b>
TAC level (ng/ml)	8.7 ± 3	8.5 ± 1.9	8 ± 3	8.1 ± 1.3	0.6693
eGFR CKD-EPI M3 (ml/min)	62.8 ± 26.3	48 ± 30	49.6 ± 20.8	58.7 ± 17.9	0.4202

IFTA, interstitial fibrosis and tubular atrophy; ATI, acute tubular injury; TCMR, T-cell-mediated rejection; AMR, antibody-mediated rejection; DSA, donor-specific antibody; BMI, body mass index; ECD, expanded criteria donor; CIT, cold ischaemia time; DGF, delayed graft function; PRA, panel-reactive antibodies; IL, interleukin; TAC, tacrolimus; eGFR CKD EPI, estimated glomerular filtration by Chronic Kidney Disease Epidemiology Collaboration.

**Table 3.** Hazard ratio (borderline changes).

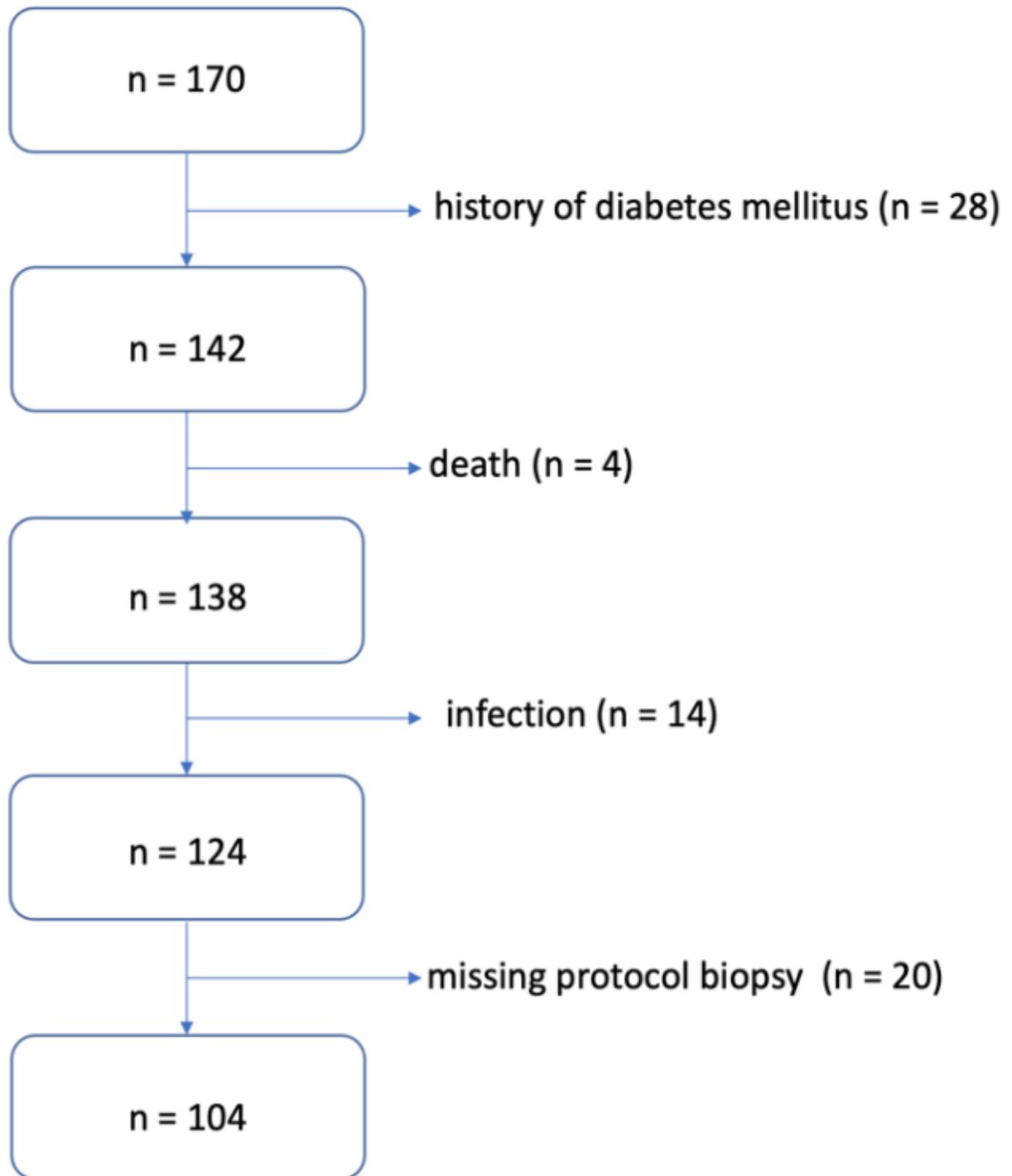
borderline	HR	95% CI	<i>P</i>
<b>Hyperleptinaemia baseline</b>	7.4979	2.4671–30.649	<b>0.0071</b>
<b>Hypoadiponectinaemia baseline</b>	3.4242	0.3087–37.9826	0.3161
<b>Hyper IL-6 baseline</b>	5.8212	0.5258–64.4488	0.1510
<b>Hypo IL-10 baseline</b>	2.0178	0.1825–22.3135	0.5669
<b>Hyperleptinaemia 3M</b>	9.7432	2.6876–8.1668	<b>0.0057</b>
<b>Hypoadiponectinaemia 3M</b>	21.3349	7.7339–67.5642	0.9743
<b>Hyper IL-6 3M</b>	2.3233	0.2097–25.7386	0.4921
<b>Hypo IL-10 3M</b>	2.8462	0.2537–31.929	0.3964

**Table 4.** Hazard ratio (DSA positivity).

DSA+	HR	95% CI	<i>P</i>
<b>Hyperleptinaemia baseline</b>	2.0444	0.9493–4.4030	<b>0.0341</b>
<b>Hypoadiponectinaemia baseline</b>	1.1807	0.4429–3.1473	0.7398
<b>Hyper IL-6 baseline</b>	0.8250	0.2970–2.2920	0.7121
<b>Hypo IL-10 baseline</b>	1.7091	0.3876–7.5360	0.4790
<b>Hyperleptinaemia M3</b>	49.8037	9.8975–69.5006	<b>&lt;0.0001</b>
<b>Hypoadiponectinaemia M3</b>	1.9938	0.8335–4.7693	0.1210
<b>Hyper IL-6 M3</b>	1.2176	0.5598–2.6487	0.6195
<b>Hypo IL-10 M3</b>	3.0746	1.0595–8.9216	<b>0.0388</b>

# Figures

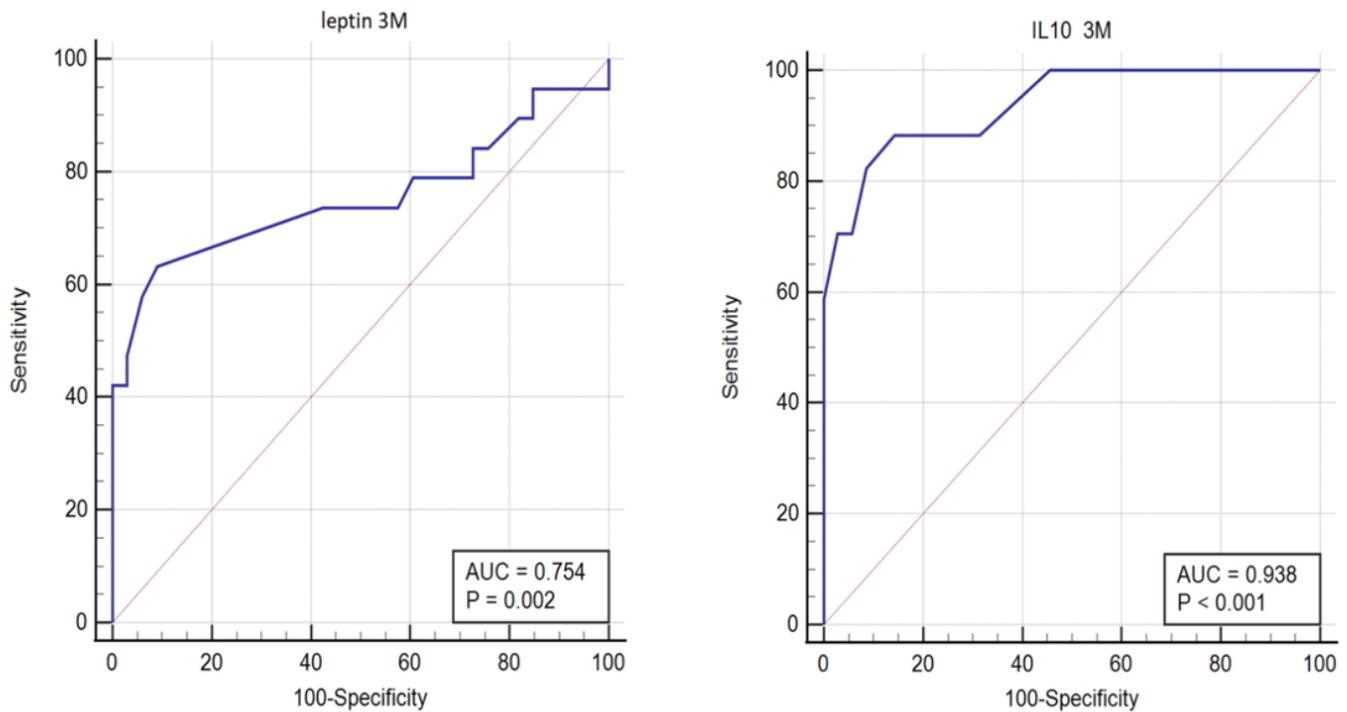
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**Figure 1**

This study was a prospective one-centre analysis that included patients (Caucasians) older than 18 years that had undergone primary transplantation of the kidney in the Martin (2018-2019), Slovakia Transplantation Center. Patients with diabetes mellitus diagnosed prior to the kidney transplantation,

patients with complicated or protracted infection, including secondary wound healing, and patients with a medical history of oncology illness were excluded from the monitoring



**Figure 2**

We added the ROC curve analysis for leptin 3M and borderline plus de novo DSA (optimal criterion = leptin level > 39  $\mu\text{g}/\text{ml}$  [specificity, 90.91%; sensitivity, 63.16%]) and for IL-10 3M and borderline plus de novo DSA (optimal criterion = IL-10 level  $\leq$  2.58  $\text{pg}/\text{ml}$  [specificity, 85.71%; sensitivity, 88.24%])