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Clinical use of an immune monitoring panel in liver transplant recipients: A prospective, observational study

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ABSTRACT

Immunosuppressive therapy greatly contributed to making liver transplantation the standard treatment for end-stage liver diseases. However, it remains difficult to predict and measure the efficacy of pharmacological immunosuppression. Therefore, we used a panel of standardized, commonly available, biomarkers with the aim to describe their changes in the first 3 weeks after the transplant procedure and assess if they may help therapeutic drug monitoring in better tailoring the dose of the immunosuppressive drugs.

We prospectively studied 72 consecutive patients from the day of liver transplant (post-operative day #0) until the post-operative day #21. Leukocytes, neutrophils, lymphocytes (CD4+, CD8+), natural killer cells, monocytes, immunoglobulins and tacrolimus serum levels were measured on peripheral blood (at day 0, 3, 7, 14, 21 after surgery). Patients who developed infections showed significantly higher CD64+ monocytes on post operative day #7. IgG levels were lower on post operative day #3 among patients who later developed infections. We also found that a sharp decrease in IgA from post operative day #0 to 3 (−226 mg/dL in the ROC curve analysis) strongly correlates with the onset of infections among HCV− patients. No specific markers of rejection emerged from the tested panel of markers. Our results show that some early changes in peripheral blood white cells and immunoglobulins may predict the onset of infections and may be useful in modulating the immunosuppressive therapy. However, a panel of commonly available, standardized biomarkers do not support in improving therapeutic drug monitoring ability to individualize immunosuppressive drugs dosing.

1. Introduction

The development of new immunosuppressive regimens greatly contributed to making liver transplantation (LT) the standard treatment for end-stage liver diseases (ESLD) [1,2]. However, immunosuppressive drugs are still burden by several side effects. In particular, over-immunosuppression in the peri-operative period after LT is associated with an increased risk of infections and sepsis, kidney injury and neurologic complications [2–4]. Clinicians carefully tailor immunosuppressive therapy to reach an optimal balance between the risk of rejection, and the risk of immunosuppressive drugs related complications in each patient. [1,3,5].

Therapeutic drug monitoring (TDM), a branch of clinical pharmacology specialized in measuring drugs blood concentration, focuses on drugs such as immunosuppressants characterized by a narrow therapeutic range [6]. TDM can thus help clinicians to “fine tune” therapy depending on the concentrations reached in the body. However, there is still controversy among clinicians whether measuring these drugs in biological fluids is a useful adjunct to their optimal administration as it remains difficult to predict and to measure the efficacy of pharmacological immunosuppression [6–8]. Therefore, new complementary and/or additional strategies to manage narrow therapeutic range drugs beyond pharmacokinetics have been explored [9–11]. In recent years, substantial progress in molecular immunology, coupled with an increasing focus on translational research, led to a growing interest in the development of assays capable of measuring the

Abbreviations: ACR, acute cellular rejection; POD, post-operative day; LT, liver transplantation; ESLD, end-stage liver disease; TDM, `therapeutic drug monitoring; CMV, cytomegalovirus; EBV, Epstein-Barr virus; NK, natural killer; Ig, immunoglobulin; BAL, bronchoalveolar lavage

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net immunosuppressive state in transplant recipients [11–15]. In this study, we used a panel of standardized, commonly available, biomarkers with the aim to explore the changes of different immunological parameters during the first 21 days after LT and assess if it may flank the TDM in modulating the dose of immunosuppressive drugs in everyday clinical practice.

2. Patients and methods

This is a monocentric, prospective, observational study that included a series of consecutive LT recipients who received an ABO compatible, whole size, liver graft. The study was performed according to the ethics guidelines of the Declaration of Helsinki. All patients were enrolled after signing an informed consent. The study was approved by local hospital ethical committee. There were no exclusions pertaining to gender or primary diagnosis.

2.1. Immune testing

The following variables were measured in peripheral blood on POD#0 3, 7, 14, 21: CD4+, CD8+, CD45+, CD64+, monocyte HLA-DR expression, natural killer (NK) cells, immunoglobulin (Ig) classes A, G, M.

2.2. Laboratory methodology

Peripheral blood samples were processed and analyzed by flow cytometry within 24 h. One hundred μl of each sample were incubated for 15 min in the dark at room temperature with the following monoclonal antibodies: anti CD45-PerCP, anti CD3 FITC, anti CD8 APC Cy-7, anti CD4 PE Cy-7. Another 100 μL of the same samples were incubated with: CD45-PerCP, anti CD64 FITC, anti-CD14-APC and anti-HLA-DR-PE. The samples were then treated with lysing solution for 10 min. All samples were analyzed on a BD FACSCANTO II using FACS-DIVA software. Up to 100.000 events per tube were acquired. Peripheral blood immunoglobulins (Ig) concentration was performed by using common techniques. All the above-mentioned tests were performed at the Central Laboratory of the Pisa University Hospital.

2.3. Concomitant treatments

Standard perioperative anti-infective prophylaxis consisted of intravenous ampicillin-sulbactam (2 g + 1 g) for two days after LT. Cytomegalovirus (CMV) and Epstein-Barr virus (EBV) viraemia were tested every seven days by real-time PCR on peripheral blood. In case of clinical signs of infection, cultures from peripheral blood, central venous catheter, urine and biliary draining were immediately collected, and chest X-ray was performed as well. Bronchoalveolar lavage (BAL) was performed to isolate the microbial agent when a pulmonary infection was suspected. Postoperative pain was controlled by intravenous morphine (1 mg/kg 40 min before the end of procedure and followed by a continuous infusion of 20–60 mg/day). According to our protocol, immunosuppressive regimen consisted of induction with anti-IL2a antibodies (basiliximab) 20 mg IV on POD#0 and 4, calcineurin inhibitor (tacrolimus) titrated to maintain blood serum levels between 6 and 10 ng/ml and antimetabolites (Mophetil Mycophenolate) 1 g twice a day. Steroid (Methylprednisolone) at an intraoperative dose of 10 mg/kg, subsequently reduced by 50% per day to a Prednisolone dose of 25 mg/day, was added in HCV negative recipients. In case tacrolimus serum levels exceeded the given threshold, its administration was hold until normalization, while it was minimized in case of clinical signs of acute kidney injury, infections or neurological impairment, *Adverse Events*.

Acute cellular rejection (ACR) was suspected when a progressive increase in transaminases was shown on POD#5 to 30 after LT, in absence of abnormal liver blood flows (US-documented), abdominal collections, potential sepsis and/or positive CMV-, EBV-, and HCV-PCR (routinely tested after LT) [16]. Primary treatment consisted of steroids boluses in HCV-RNA negative patients and CNI dose adjustments in HCV-RNA positive recipients [17]. Whenever clinically indicated (e.g.: difficult differential diagnosis, steroid resistant rejection, etc.), ACR was confirmed histologically and graded according to the Grading for Acute Liver Allograft Rejection and expressed as Rejection Activity Index (RAI) [16,18]. In this case, rejection episodes were treated if RAI ≥ 7. Infections were defined according to the Centers for Disease Control and Prevention definitions [19]. Sepsis was defined as a systemic response to infection manifested by systemic inflammatory response syndrome [20] and deemed severe in presence of organ dysfunction or when requiring hospitalization.

2.4. Statistical analysis

Categorical data were described by frequency, whereas continuous data by median. Several “delta” variables, assessing immune response, were calculated between the time-points of the study. Kolmogorov-Smirnov test was performed to evaluate the normality of quantitative data distributions. Comparison between qualitative variables was performed using chi-square test; whereas, quantitative variables (“delta”) were analyzed with Mann-Whitney test (two-tailed). Finally, a ROC analysis was performed to identify the best cut-off value for the “delta” values showing a correlation with the clinical outcome. The “area under the curve” was analyzed with a non-parametric test. Differences were considered significant at $p < 0.05$. All the descriptive and inferential analyses were carried out by SPSS® v.24 (IBM) and Prism® v. 7 (GraphPad Software) technologies.

3. Results

3.1. Clinical features

Seventy-two consecutive patients (53 men, 19 women) who underwent LT were included in the study. The median age at LT was 54.1 ± 8.9 years (range 30–66). Forty-five patients underwent transplant for non-HCV related causes (14 HBV related cirrhosis, 18 ethanolic related cirrhosis, 13 other causes) and 27 patients for HCV-related cirrhosis. After LT, 26 patients (36%) developed infection (18 HCV+, 8 HCV−): 14 patients developed infection on POD#7 (53.8% of the infections), 9 on POD#3 (34.6%), 2 on POD#14 (7.7%) and one patient on POD#1 (3.9%). The urinary tract was the most common site of infection (9 patients). Five patients had infection of the soft tissues, and five patients developed pneumonia (diagnosed by chest X-ray and BAL). Three patients presented primary blood stream infections (two of them from the central venous catheter) and 2 patients had biliary infections. One patient presented CMV reactivation, and one had colitis. The etiologic microbiological agent was isolated in 16 out of 26 cases (61.5%): *Pseudomonas aeruginosa* in 4 cases; *E. coli* in 4 cases; *A. baumanii*, *C. difficilis, CMV, E.faecalis, Enterobacter, Klebsiella pneumonia, Morganella Morganii* and *Staphilococcus epidermidis* in one each. Eight patients (30.8% of infected patients, all HCV−) developed sepsis or septic shock: one patient died due to sepsis on POD#14. Finally, a graft's rejection was diagnosed in 10 patients, 7 (15.6%) in HCV negative recipients and 3 (11.1%) in HCV positive recipients. In 6 cases steroid boluses were administered. In one case a liver biopsy was required to differentiate rejection to HCV recurrence (severe rejection).

3.2. Kinetics of circulating leukocytes

We divided the patients into two groups: HCV+ and HCV− patients due to the biological difference of the underlying disease and the intraoperative use of steroids We compared the absolute values of every immunological variable between the two groups at each time-point. The absolute leukocyte count was normal in both groups on POD#0.

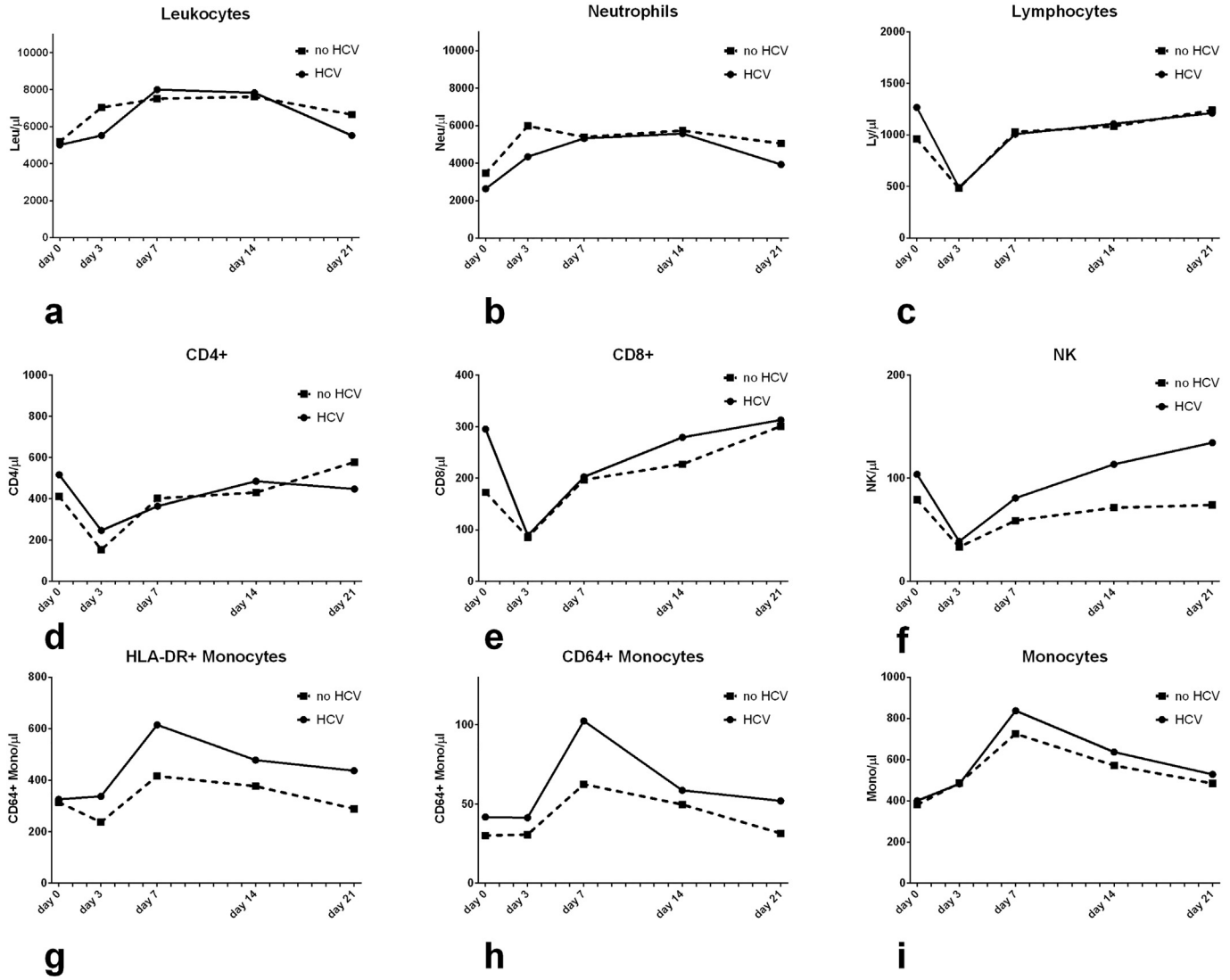

Fig. 1. Trends of leukocytes after liver transplant. No differences were observed between HCV+ and HCV− patients. The absolute leukocyte number (a) and the neutrophils (b) showed an increase at day +3 more marked in HCV− patients. The lymphocytes (c, d, e) and the NK cells (f) showed a nadir at day +3 and a slow recovery to the pre-transplant levels at day +21. The monocyte populations (g, h, i) showed an increase from day 0 to day +7 and then a decrease.

Total circulating leukocytes increased from POD#0 to 7 (Fig. 1a), with no differences between the two groups at any time-points. After POD#7, total leukocyte count remained stable in HCV− group until POD#21; whereas, it slightly decreased in HCV+ group within the same time frame. Neutrophils levels showed a similar trend of total leukocytes. Lymphocytes (Fig. 1c) decreased in both groups from POD#0 to 3; and on POD #7, the absolute lymphocyte count came back to POD#0 level. CD4+ and CD8+ circulating lymphocytes presented the same trends of total lymphocytes (Fig. 1d and e). NK levels followed the same trend in both groups as well, although the recovery in HCV+ patients was more marked on POD#7. Monocytes showed normal counts on POD#0 in both groups (Fig. 1g); on POD#7 monocytes reached their highest levels in both groups; after POD#7, monocytes presented a progressive decrease in their number until POD#21. The absolute numbers of HLA-DR+ (Fig. 1h) and CD64+ (Fig. 1i) monocytes were stable from POD#0 to 3 in both groups; then, an increase of both populations on POD#7 was observed, more pronounced in HCV+ group. The proportion of HLA-DR+ monocytes was close 80% in both groups on POD#0; then, it remained around 70% on POD#3 and 7 in HCV+ group; whereas, it decreased respectively at 48% and 57% in HCV− patients. CD64+ monocytes were at similar levels in both groups on POD#0 and remained stable on POD#3. On POD#7, the basal value increased 2.5-fold in HCV+ and 2.0-fold in the HCV− group. The percentage of CD64+ monocytes remained stable at 7% of total monocytes in HCV+ patients; whereas, it reached 14% of total monocytes on POD#7 in HCV− group.

3.3. Kinetics of the serum immunoglobulins

The basal levels of Ig were normal for all three main classes of serum immunoglobulins (IgG, IgA and IgM); all Ig presented a decrease from POD#0 to #7 in either HCV+ and HCV− groups. Regarding IgG levels (Fig. 2a), HCV+ patients showed a trend toward the recovery of basal levels on POD#21, which was different from that observed in HCV− group, although not statistically significant. IgA (Fig. 2b) and IgM (Fig. 2c) showed a reduction from the basal levels on POD#3 in both groups; then, both slowly increased in the two groups without any difference.

3.4. Tacrolimus serum levels

Tacrolimus (FK) was administered to all patients from POD#0 and dose titrated to maintain serum levels between 6 and 10 ng/ml. The median FK serum levels slowly increased from POD#0 to 21 in both

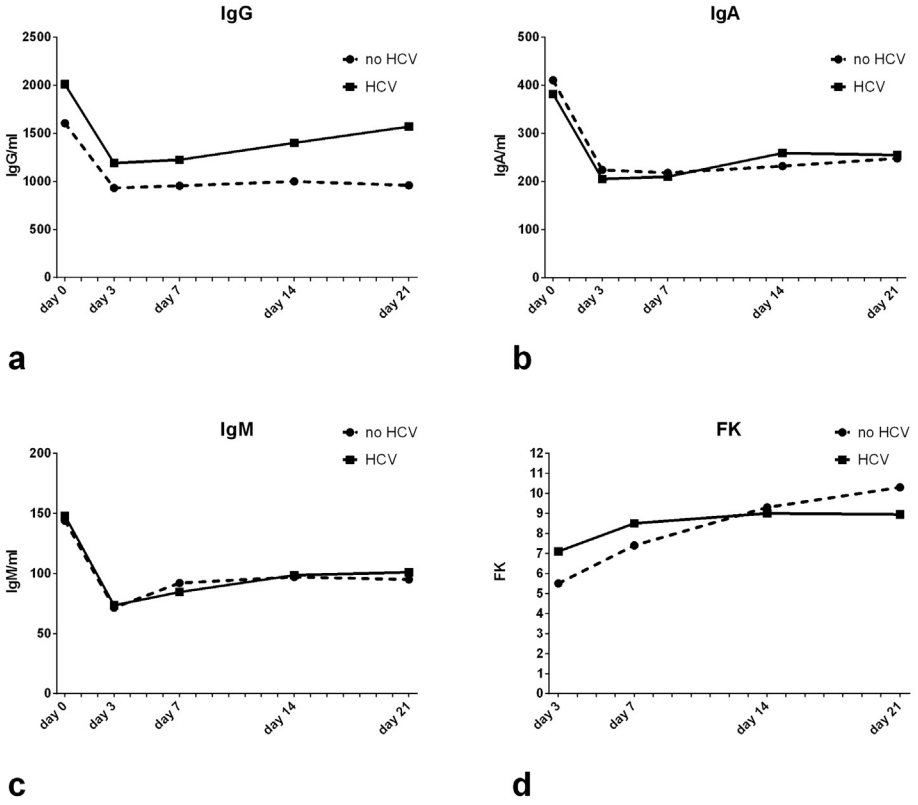

Fig. 2. Immunoglobulins (a, b, c) showed a rapid decrease from day 0 to day +3 for the three classes (IgG, IgA and IgM) and then they remained at stable levels until day +21. The levels of serum tacrolimus (FK) did not differ between HCV+ and HCV− patients after day +3 (f).

groups (Fig. 2d), without any statistical differences between HCV+ and HCV− patients.

3.5. Patients who developed infections showed peculiar immune features in the first 7 days after LT

We compared the absolute values of all the above-mentioned parameters at each time-point and their variations ("delta") from POD#0 to 3 and from POD#3 to 7 between infected and not infected patients, in order to evaluate possible variations or differences with a predictive impact on infection onset in the first 7 days after LT. First, we compared the rate of infections documented in HCV+ (8 out of 26) and HCV− (18 out of 45) patients, and no significant differences between the two groups emerged from this analysis ($p = 0.76$). Then, we compared the absolute values of all immunological parameters we measured within patients who developed infections and patients who did not have any evidence of infections at each time-point. Herein, we report only the significant differences. The median absolute number of circulating leukocytes was higher on POD#3 ($p = 0.012$) and on POD#7 ($p = 0.001$) in those patients who developed infections if compared to uninfected patients. In particular, neutrophils ($p = 0.009$ and $p = 0.016$, on POD#3 and 7 respectively) were higher in patients who developed infections (Fig. 3a). CD64+ monocytes were significantly higher in infected patients on POD#7 ($p = 0.029$) (Fig. 3b). IgG levels on POD#3 were significantly lower in patients who presented infections ($p = 0.049$) (Fig. 3c), whereas higher IgM levels on POD#0 showed a trend of correlation with the subsequent onset of infections ($p = 0.086$). A lower level of serum FK on POD#0 seemed to be correlated with higher rate of infections ($p = 0.031$). The neutrophil to lymphocyte ratio on POD#0, reported as a precocious marker of immunosuppression after LT in other papers [21] did not show any correlation with the onset of infections in our series.

3.6. The variations of some immunological parameter ("Δs") within the precocious timepoints were correlated to the onset of infections in HCV− patients

We calculated increase and decrease ("Δ") of the immunological parameters through the timepoints from POD#0 to 3 ("Δ 0–3"), from POD#0 to 7 ("Δ 0–7"), and from POD#3 to 7 ("Δ 3–7") in those patients who developed infections and those who did not; then, we compared those "Δs" between these two categories of patients, in order to verify the impact of those variations on the risk of infection. We found a difference between HCV+ and HCV− patients (Table 1): in HCV+ patients, only the variation of monocytes from POD#3 until POD#7 showed a near-significant trend of correlation with the onset infections ($p = 0.088$) when compared to uninfected patients. In contrast, it was possible to evidence differences in many "Δs" between infected and uninfected patients in HCV− patients. A marked decrease in IgA levels from POD#0 to 3 ("ΔIgA 0–3") was correlated with the onset of infections ($p = 0.019$) (Fig. 3d and e). In contrast, IgA levels increased from POD#0 to 7 in patients who developed infections ($p = 0.035$); whereas, there was a negative "Δ" of IgA in infected patients within the same timepoints. The monocytes increased in either infected and uninfected HCV− patients from POD#3 to 7 ("ΔMonocytes 3–7"), but the increase was significantly higher ($p = 0.042$) in HCV− patients who experienced infections on POD#7; a similar near-significant ($p = 0.063$) trend, was detected for CD64+ monocytes in the same period. Although high levels of Procalcitonin (PCT) are commonly used in clinical practice, variations in PCT did not seem to be specifically predictive of infections. Interestingly, the increase of FK serum levels from POD#0 to 3 ("ΔFK 0–3") was more marked in HCV− patients who developed infections ($p = 0.121$).

The same statistical analyses used in the prediction of infections was applied to graft rejection. All patients who presented graft rejections were HCV−, which was significantly higher ($p = 0.031$) when

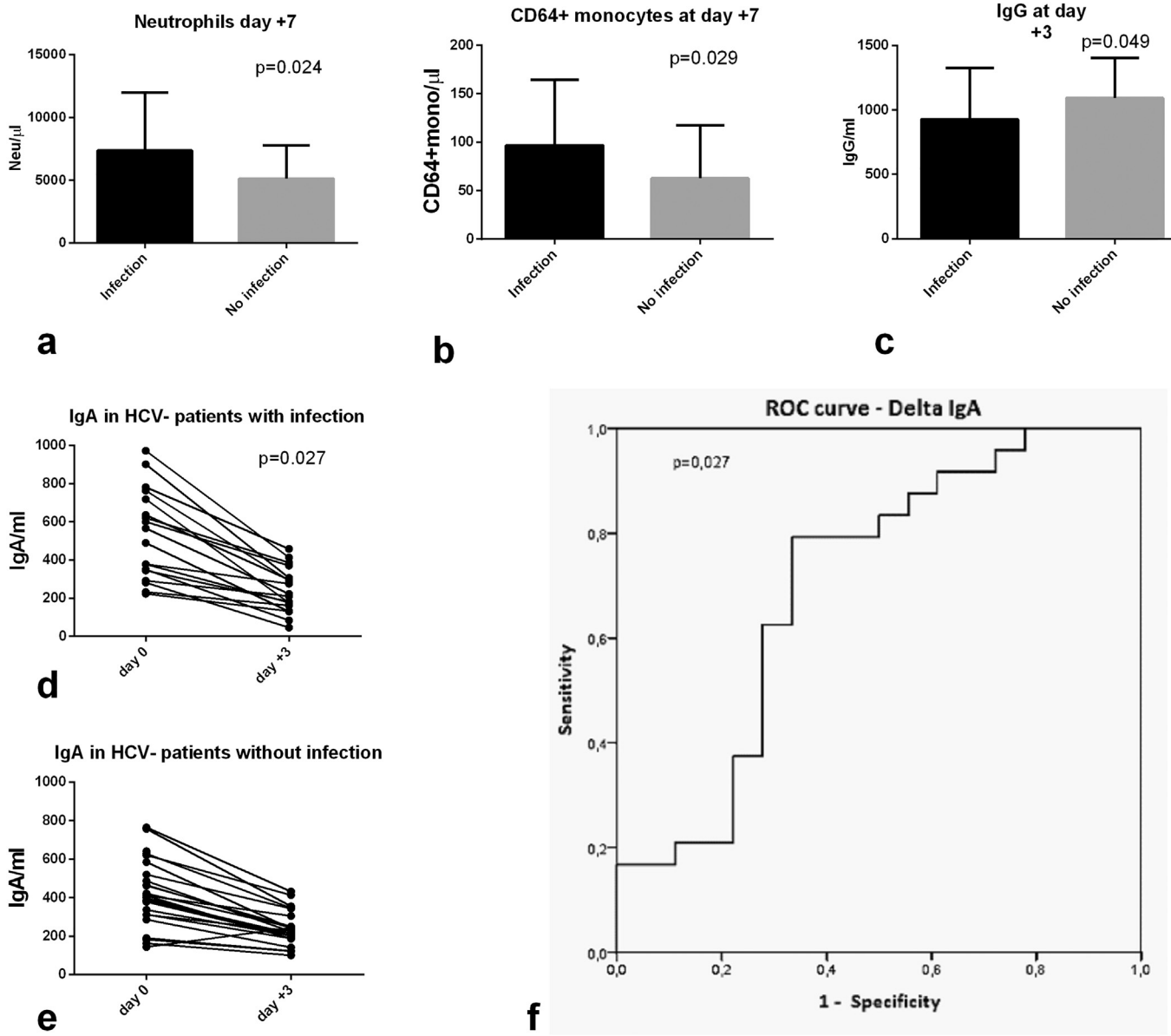

Fig. 3. Mann-Whitney tests: patients who developed infection showed a significantly higher level of neutrophils (a) and CD64+ monocytes (b) at day +7 and a lower IgG level at day +3 (c). The IgA levels decreased significantly at day +3 in HCV− patients who presented infections at day +7 (d), whereas they showed a less marked decrease in HCV− patients who did not develop any infection (e). The ROC curve (f) shows that a reduction of IgA levels of −226 mg/dL (sensitivity 79%, specificity 67%) from day 0 until day +3 is able to predict the onset of infection at day +7 after transplant in HCV− patients.

compared to the group of HCV+ patients. Only a significant higher level of neutrophils on POD#7 ($p = 0.043$) was detectable in those patients who rejected the graft when compared to the other patients. Moreover, FK levels ($p = 0.07$) and total leukocyte count ($p = 0.092$) on POD#7 were near-significantly higher in patients who developed graft rejection. All the "Δs" were not significant for the prediction of rejection, although "ΔIgA 3–7" showed an increasing trend in those patients who rejected the organ. The onset of both infection and rejection occurred in 5 over 7 patients; in light of that, we performed a chi-square test, showing a near significant concordance ($p = 0.065$) between the onset of graft rejection and infections.

Finally, since most of the measured parameters showed a significant association with both infection and rejection occurrence, we focused our attention on those statistical significances that appeared to be related with only one of the two examined clinical conditions. Lacking specific variables predicting the onset of graft rejection, we performed a ROC curve analysis on those parameters associated only with the onset of infections. We analyzed the following values: CD64+ monocytes on POD#7; IgG levels on POD#3; "ΔIgA 0–3"; "ΔMonocytes 3–7". The analyses showed reliable values only for "ΔIgA 0–3": the ROC curve (Fig. 3f) showed a well-defined cut-off between infection and no-infection at −226 mg/dL of IgA (sensitivity 79%, specificity 67%).

4. Discussion

The postoperative course following LT can be still frequently affected by complications including infection and rejection suggesting an inadequacy in current immune function monitoring [13]. However, monitoring the immune system after LT remains difficult and the clinical judgement of an expert transplant clinician for pooling together laboratory data in order to and individualize immunosuppression therapy still plays a pivotal role. Thus, an unmet need exists to measure immune function and assess the risk of clinical complications objectively [22]. Many options for immune monitoring have been considered

Table 1
Impact of changes in immunological parameters ("delta" values) in predicting the occurrence of infection after liver transplant. The "Delta" values of the immunological parameters were calculated as the differences through the timepoints from day 0 until day +3 ("Δ 0–3"), from day 0 until day 7 ("Δ 0–3") from day and from day +3 until day +7 ("Δ 3–7") and were expressed as positive or negative values. The Mann-Whitney test were performed by comparing the median "delta" values of each parameter between patients who developed infections and patients who did not develop any infection. Units of measure: IgG, IgA, IgM = mg/dL; Monocytes, CD64+ Monocytes, = cells/mcL. Significant P value < 0.05; FK = ng/mL; PCT = ng/mL.

	Delta (Δ)	P-Value of Mann-Whitney test (infected vs non infected)	Median Δ of infected patients	Median Δ of non infected patients
All pts	Δ Mono 0–3	0,064	385,7	176,5
	Δ CD64 0–7	0,064	56,1	23,1
	Δ IgA 0–3	0,140	−235,1	−178,5
	Δ IgA 0–7	0,266	−227,1	−210,1
HCV+	Δ Mono 3–7	0,100	555,5	267,7
HCV−	Δ Mono 0–3	0,151	244,1	118,8
	Δ CD64 0–7	0,105	48,1	26,1
	Δ CD64 3–7	0,095	66,2	38,1
	Δ IgA 0–3	0,027	−267,1	−175,5
	Δ IgA 0–7	0,079	−261,1	−176,1
	Δ IgA 3–7	0,057	22,1	−13,1
	Δ FK 0–3	0,121	3,1	2,1
	Δ PCT 3–7	0,096	−1,8	−0,2

so far. However, no single method or assay proved to be able to meet the diagnostic requirements while answering the basic technical requirements: an assay that is standardized, reproducible, cost-effective, easy and intuitive to perform [22]. Most vary in degree of promise based on ease of execution, precision, specificity, reproducibility and cost, as well as the type of information they provide. Many assays have been tested in the research setting to identify possible biomarkers that may be used to predict significant clinical events such as acute cellular rejection and therefore allow modification of patient's immunosuppressive regimen prior to a clinical event [13]. However, these generally require significant laboratory processing and have had difficulty becoming established in common clinical use outside the research setting. Finally, it is possible that multiple assays or a combination assay may show useful in the same patient at different times to distinguish an accurate immunological profile [23]. Therefore, in this study, we aimed to see if the use of a panel of standardized, commonly available and relatively inexpensive biomarkers can help clinicians to better individualize immunosuppressants administration after LT and flank TDM in everyday clinical practice.

The global view of trends in immunological variables in our patients is summarized in Fig. 4. The trend of the neutrophils was the same either in HCV− and HCV+ patients, though neutrophils were higher in HCV− patients on POD#3 and 21. This finding could be explained by the high dose of steroids used in the latter subset. The significant higher level of neutrophils in infected patients is also an expected finding, but neutrophils are unfortunately also involved in the ischemia-reperfusion injury, one of the mechanisms able to trigger graft rejection [24]. Consequently, it is not surprising that neutrophils levels on POD#7 were also significantly higher in patients who developed rejection. Neutrophils-to-lymphocytes ratio (N/Rr) was used as a predictor of mortality in many reports of liver surgery and LT [21,25,26]. In our series, the N/Rs at every time-point did not correlate with the onset of infection and rejection. Regarding the monocytes, their higher levels on POD#7 in all the patients who developed infection, and the marked "Δ Mono 3-7" in the HCV− patients with infections, are consistent with their role as acute inflammatory cells. The interaction between monocytes and Th1 lymphocytes in promoting the onset of de-novo autoimmune hepatitis after LT has been previously described [27]. Based on the analysis of our data, a more specific role may be assigned to the monocytes as markers of infection, because their variations are not significant among patients who rejected the graft. The expression of CD64 on the monocytes [28] has been previously related to the development of infections after LT, and this finding was confirmed in our data series on POD#7. The CD4+ and CD8+ lymphocytes, and the natural killer (NK) cells are the most important immune cells involved in the recognition of the non-self. The circulating levels of NK cells, and CD4+ and CD8+ lymphocytes showed a rapid decrease on POD#3 in either HCV+ and HCV− groups, with a following recovery at pre-transplant levels on POD#7; this kinetic may reflect the in vivo effect of anti-IL2a antibodies against T-lymphocytes [29,30]. We did not find any statistical difference between HCV+ and HCV− patients in circulating levels of NK, and CD4+ and CD8+ lymphocytes, and they did not seem to show any correlation with either infection and rejection. Then, substantial evidence exists regarding the role of specific lymphocyte populations in the development of graft rejection and tolerance [31]. For example, several papers [32–34] demonstrated the role of T

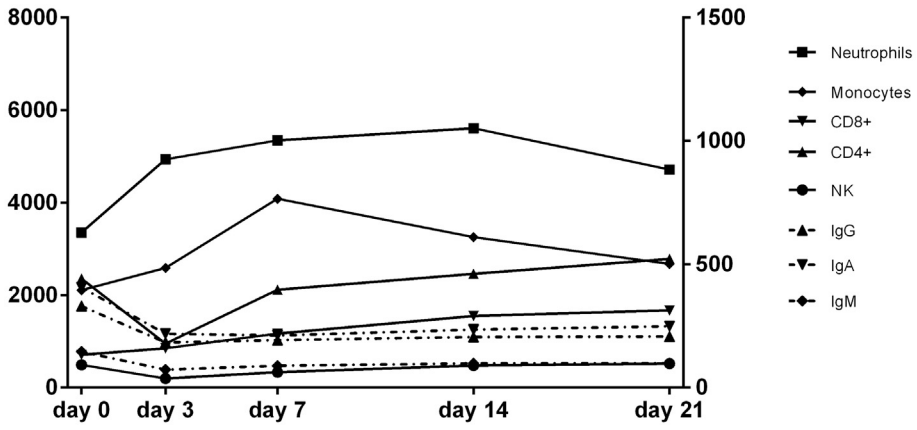

Fig. 4. Overview of the variations in the immunological parameters in the first 21 days after liver transplant. Neutrophils, monocytes and IgG are plotted on the left y axis; CD4+, CD8+, natural killer (NK), IgA and IgM are plotted on the right y axis. The values are considered as cells/μl of peripheral blood for what concerns neutrophils, monocytes, CD4+, CD8+ and NK; whereas, the values must be considered as mg/ml of peripheral blood for IgG, IgA, and IgM.

CD4 + FOXP-1 + -regulatory cells (Tregs) in the induction of tolerance [35]. However, other populations of CD4 + and CD8 + lymphocytes are able to recognize antigens on the surface of transplanted hepatocytes [36], and the NK cells have also been described as potential effectors of rejection [37,38] with a specific role described for donor-derived CD56-Dim NK cells after LT [39]. The in vivo study of lymphocytic subpopulation's kinetics would need a broader flow-cytometric panel; a more detailed study is needed to develop a tailored approach to immunosuppressive therapy and will be the object of further studies. The lower incidence of rejection in HCV + patients and the association of specific "Δs" with the onset of infections only among HCV − patients probably reflect a different immune status of the two groups. HCV infection causes the alteration of many immunological parameters, an exhaustion and a skew in lymphocytes functions toward the immune regulatory functions [40]. The HCV-related immune impairment may explain the lower rate of acute rejection because of the minor alloreactivity, as demonstrated in other studies in patients who received LT in course of chronic viral infections [41]. Regarding the immunoglobulin kinetics, hypogammaglobulinemia is an important risk factor for infections in post-transplant [42]. Basiliximab has been previously reported as cause of hypogammaglobulinemia after intestinal transplant [43] and it seemed to be associated with a worse prognosis [44]. Nevertheless, other causes of decrease in immunoglobulin levels have been described, such as blood and drain losses [45]. In our series, there were no patients who developed severe bleeding or drain loss after surgery. The marked decrease of IgA on POD#3 in HCV − patients who developed infections ("ΔIgA 0–3") may reflect a deeper immunosuppression. IgA are the most represented isotype in the lumen of gut, respiratory system and urinary tract, and they play a role in limiting the proliferation of pathogens and bacterial translocation [46]. The results of the ROC-curve indicate that patients with a decrease of serum IgA > 226 mg/dL show high-risk of infections. The persistent decrease in immunoglobulin levels after treatment with monoclonal antibodies is a common collateral damage after treatment with Basiliximab [47]. Regarding the selective IgA deficit, a recent study showed that the commonly used antibiotics may modify the microbiota and lead to IgA depletion [48]; the authors reported an increased susceptibility to pulmonary infections by *Pseudomonas aeruginosa,* and this finding seems confirmed in our series, where pneumonia was the most common infection (five cases), and *Pseudomonas aeruginosa* had the highest incidence of isolation (four patients). The contemporary higher increase of FK serum levels from POD#0 to 3 ("ΔFK 0–3") in HCV − patients who developed infections (although not statistically significant) suggests that a careful evaluation of the total dose of immunosuppressive therapy in the first 3 days after LT may prevent the onset of infections in patients pre-treated with Basiliximab. This time frame is also crucial in the development of graft rejection. Acute cellular rejection remains an important source of morbidity after LT, particularly if rejection is moderate or severe. Since liver biopsies are seldom performed, diagnostic noninvasive markers would be useful. Currently, many tools are available to predict the potential risk of graft rejection [13]. Many of them, such as the mixed lymphoid reaction (MLR) [49], the Limiting dilution assays (LDA) [50] and the Enzyme-linked immunosorbent spots (ELISPOT) [50] are antigen-specific techniques, but their use limited by the difficulty of obtaining donor-derived peripheral blood cells from orthotopic donors. ImmunKnow (Cylex Ltd., United States) was developed as a biomarker to guide immunosuppressant dosing following solid organ transplantation [51]. Unfortunately, studies in LT recipients reported contradictory results in predicting acute rejection and infection and limited its widespread use in clinical practice [52–54]. Even if the biological activity of each individual drug could be accurately determined, this would not provide an objective net biomarker of immune function since the cross-reactive effects of the drugs would remain uncertain. Moreover, with the increased use of antibody therapies, drug monitoring has become even more challenging. As such, TDM may continue to assist clinicians in managing patients, but it is unlikely to be the dominant method for future immune system monitoring following LT [13].

In conclusion, our results confirm that the peripheral blood white cells and immunoglobulins experience changes after LT. Some of these changes, such as a marked decrease of IgA and IgG immunoglobulins and an increase of circulating monocytes on POD#3, seem to predict the onset of infections. Other changes appear to be involved either in infection and in rejection, such as the neutrophils POD#0 and 7 after LT. However, a panel of commonly available, standardized biomarkers was not of great support at the bedside to improve TDM's ability to individualize immunosuppressive drugs dosing.

Declarations of interest

None.

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