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Clinical significance of serum hepcidin-25 levels in predicting invasive fungal disease in patients after transplantation

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Abstract. – BACKGROUND: Currently, it is important to identify a good biomarker to predict treatment-related complications in patients with transplantation. This study aimed to evaluate the significance of serum hepcidin-25 in predicting invasive fungal disease (IFD) after transplantation.

PATIENTS AND METHODS: A total of 57 patients who underwent transplantation were included in this study, and their serum samples were obtained and stored at –80°C for analysis. The serum hepcidin-25 were assayed using enzyme-liked immunosorbent assay (ELISA), and hypersensitive C reactive protein (hsCRP) and 1,3-beta-D glucan were measured using standard laboratory techniques. These indices were monitored weekly, from one week before transplantation to four weeks after transplantation.

RESULTS: The median pretransplant serum hepcidin-25 level was 37.00 ng/mL which was higher than that of healthy volunteers ($p < 0.001$). Because the higher hepcidin-25 level of the third tertile among the patients was 39.855 ng/mL, we set a cutoff level of 40 ng/mL to divide them into low- and high-hepcidin-25 groups (n = 38 and 19, respectively). The prevalences of the documented infection in the two groups were 2.6% and 26%, respectively ($p = 0.019$). The high-hepcidin-25 group was monitored after transplantation. The hepcidin-25 level peaked one week after transplantation, followed by gradual decrease. The plasma (1-3)-beta-D-glucan reached the summit two week. The proven of IFD was delayed 10 days on average after hepcidin-25 had arrived summit and 5 days after (1-3)-beta-D-glucan peaked.

CONCLUSIONS: The pretransplant serum hepcidin-25 level would be a useful indicator for predicting the risk of infection after transplantation; and the dynamic changes of hepcidin-25 in patients with high-hepcidin-25 group would help to predict IFD after transplantation.

Key Words:

Hepcidin-25, Invasive fungal disease, Transplantation, Prognosis.

Introduction

Tumor has been the leading killer of human health. Transplantation has been widely performed as a potentially curative treatment for intractable tumor malignancies with conventional chemotherapy. However, despite recent advances in the treatment of infectious disease and conditioning regimens for transplantation, treatment-related complications remain a major problem. Therefore, it is particularly important to identify a good biomarker that can predict treatment-related complications before transplantation and monitor dynamic drift. A recently accumulated body of evidence suggests that iron overload is associated with adverse clinical outcome[1,2]. Other studies have shown that pretransplantation iron overload in autologous or allogeneic hematopoietic stem cell transplantation (SCT) was a risk factor associated with posttransplant complications, such as mucositis, bacterial, fungal infection, and hepatic veno-occlusive disease (VOD)[3-6]. Invasive fungal diseases (IFD) are a major cause of morbidity and mortality in patients with transplantation. Their incidence has risen dramatically in recent years. The diagnosis of IFDs remains difficult, even if the European Organization for the Research and Treatment of Cancer (EORTC)/Mycosis Study Group (MSG) criteria are applied for study purposes to classify the likelihood of these infections[7].

Hepcidin is the key regulatory protein in iron homeostasis. Its synthesis is attenuated in hereditary hemochromatosis and elevated in anemia of inflammation[8,9]. The peptide promotes internalization and degradation of ferroportin, the sole mammalian iron exporter.With this functionality, hepcidin negatively regulates iron absorption from the intestine and iron release from recycling macrophages and hepatic stores. Three isoforms of hepcidin, namely hepcidin-20, hepcidin-22 and

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hepcidin-25, have been identified, which are composed of 20, 22 and 25 residues respectively. However, only the 25-residue isoform promotes ferroportin internalization and degradation, the well-characterized central role of hepcidin in iron metabolism[10].

We hypothesized that serum hepcidin-25 level could be a useful predictor of iron overload and inflammatory condition prior to transplantation. Furthermore, hepcidin-25 could monitor dynamic transformation of infection and predict infection after transplantation.

Patients and Methods

The study group was comprised of 57 consecutive patients (31 males and 26 females, mean age 49 (25-65) years) at Sichuan Academy of Medical Science and Sichuan Provincial People' Hospital from October 2009 to January 2010. There were 27 patients with haematologic tumors suffering autologous or allogeneic SCT, 18 patients undergoing liver transplantation and 12 patients experiencing kidney transplantation. The normal control group was composed of 50 healthy volunteers that did not present anemia or inflammation. All the patients provided their written informed consent.

Before the administration of conditioning regimens, at approximately 8 am serum samples were obtained and stored in tubes at –80°C until analysis. The levels of serum hepcidin-25 were assayed using enzyme-liked immunosorbent assay (ELISA) kit (DRG, Marburg, Germany). Other serum parameters were measured using standard laboratory techniques, such as serum ferritin, iron, hsCRP (hypersensitive C reactive protein) and 1,3-β-D glucan. These indices were monitored weekly, beginning from one week before transplantation to four weeks after transplantation.

The diagnosis of invasive fungal disease was made according to EORTC and IFICG definitions[7]. The IFD was diagnosed comprehensively by host factor, clinical symptoms, microbiology and histopathology. Grading diagnosis was used, that's proven, probable and possible. Endpoints included cumulative incidences of documented fungal infection.

Statistical Analysis

All data were managed and analyzed using the SPSS version 15.0 software (SPSS Inc., Chicago, IL, USA). Quantitative data were presented as median and range. Overall survival rate was estimated using Kaplan-Meier methods. A p-value of less than 0.05 (two-tailed) was considered to indicate a significant difference.

Results

Characteristics of Patients and the Cumulative Incidence of IFD-Related Mortality

As compared to healthy control volunteers, the serum levels of both ferritin and hepcidin-25 were significantly increased, while the serum level of iron was significant decreased. The median pre-transplant serum hepcidin-25 level was 38.31 ng/mL (range: 17.01-73.96 ng/mL). It's higher than that of healthy control volunteers (median 18.70 ng/mL, range 5.51-27.35 ng/mL, n=50) ($p < 0.001$). Because the higher hepcidin-25 level of the third tertile among the patients in this study was 40.86 ng/mL, we set a cutoff hepcidin-25 level of 40 ng/mL for practical use to divide the patients to low- and high-hepcidin-25 groups (n = 38 and 19, respectively). The two groups were followed up in 4 weeks after transplantation. In the follow-up, invasive fungal disease was regarded as dead event. The cumulative prevalences of the documented infection in the two groups were 5.26% for low-hepcidin-25 group and 26.32% for high-hepcidin-25 group, respectively; and the degree of infection was also serious in high-hepcidin-25 group. And the result from Figure 1 showed that the difference in cumulative prevalence of infection-related mortality between two groups was statistical significantly ($p = 0.019$) (Figure 1).

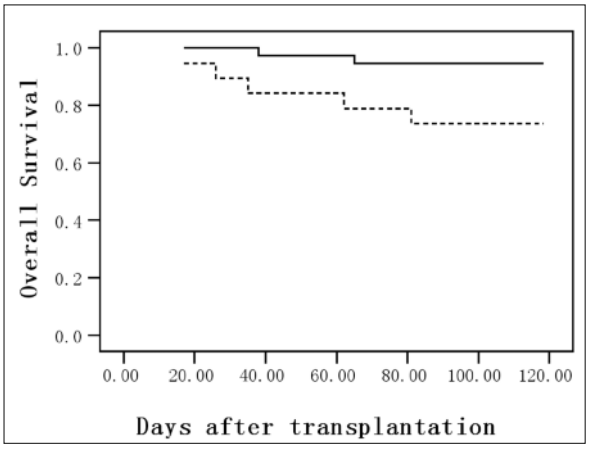

Figure 1. Overall survival rate (OS) after transplantation. Solid black line, the low-hepcidin-25 group (< 40 ng/mL); solid gray line, the high-hepcidin-25 group (≥ 40 ng/mL).

Dynamic Changes After Transplantation in High-Hepcidin-25 Group

As compared to low-hepcidin-25 group, the speed of hematopoietic recovery was lower in high-hepcidin-25 group; and high serum ferritin and low iron was also significant in high-hepcidin-25 group. In the high-hepcidin-25 group, the changes of the parameters involved in infection during transplantation were monitored and shown in Figure 2. The serum hepcidin-25 level at one week before transplantation was evidently higher (median 51.82 ng/mL) than that in the control group (median 18.70 ng/mL) ($p < 0.001$); it further increased after transplantation. The mean hepcidin level peaked one week after transplantation (median 129.60 ng/mL), followed by gradual decrease until four weeks (Figure 2A). The plasma (1-3)-β-D-glucan level which used as a predictor of documented invasive fungi infections reached the summit two week after transplantation (median 123.73 pg/mL normal range, < 10 pg/mL) and dropped by degrees with conditioning infections cure in majority of the cases (Figure 2B). In almost all cases, the serum hsCRP level was elevated one, two and three weeks after transplantation (three week; median 160.22 μg/mL; normal range, 0-3 μg/mL). The probable fungi invasive infections was diagnosed behind 8 days, when hepcidin-25 summit. It's 4 days after plasma (1-3)-β-D-glucan peaked.

Discussion

In the last nearly 20 years invasive fungal infections have assumed to be increasing trend. A great series of epidemiological studies made by American Centers for Disease Control and Prevention have shown that the morbidity of invasive fungal infection was 178.3 per million annually and the mortality was 29% 40%[11]. Invasive fungal infection could make a big poor effect on

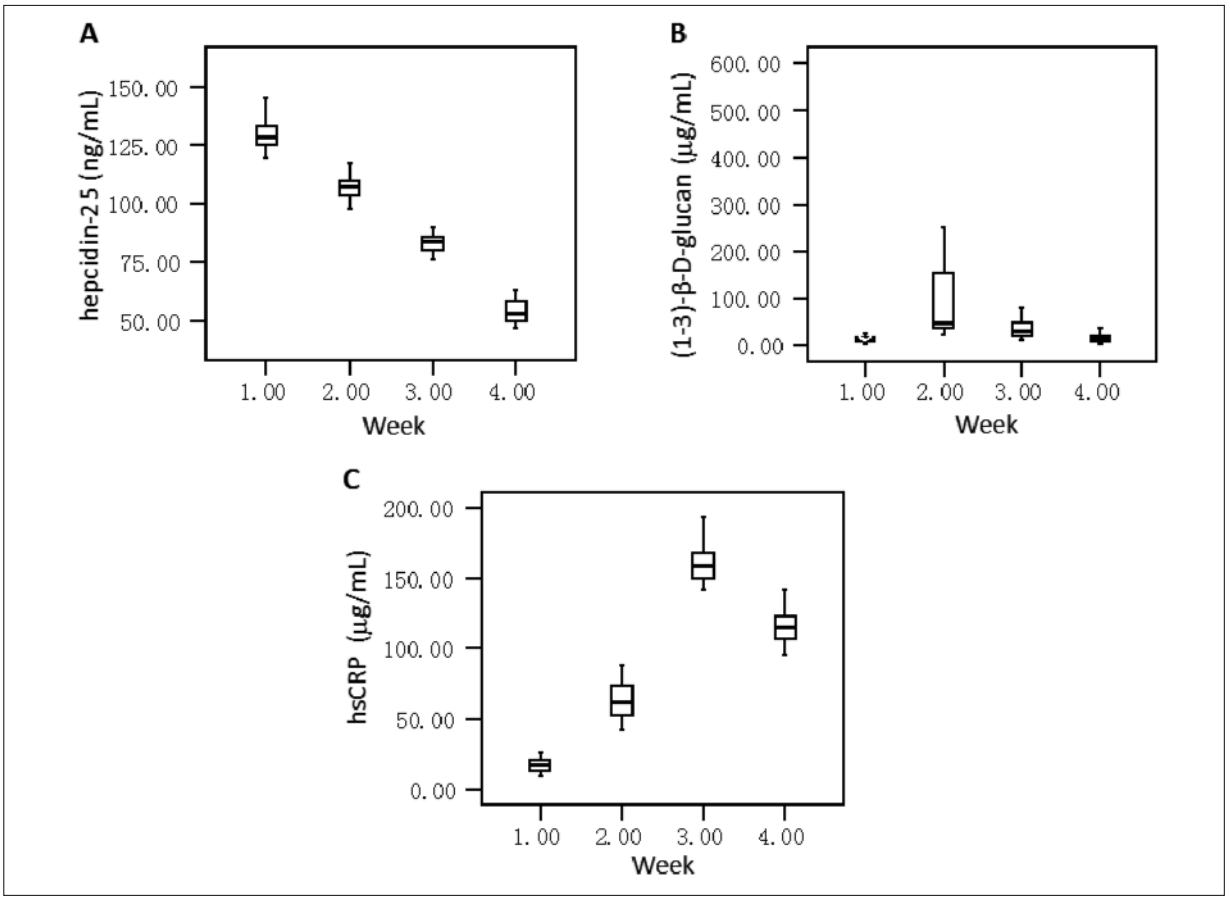

Figure 2. Sequenctial changes in each parameter after transplantation. Dynamic variations in hepcidin-25, (1-3)-β-D-glucan, hsCRP are shown in box plot. Each box extends from the first to third quartiles. The median values are shown as bars in the middle of the boxes and the end of the whiskers indicates the upper and lower values.

prognosis, especially in transplant patients. In the study, we found and demonstrated an association between the pretransplant serum hepcidin-25 levels and the cumulative incidence of IFD after transplant. The findings suggest that the pretransplant serum hepcidin-25 level could be regarded as a good index to predict infection after transplantation.

As a new acute inflammatory response protein, Hepcidin-25 participates in body reaction to anemia, anoxia and inflammation. As a negative regulated hormone of iron metabolize, hepcidin-25 plays an important role in inherent immunity, chronic inflammation anemia. Hepcidin is considered to be a central molecular regulating iron metabolism. It decreases iron absorption in the intestine and blocks iron release from its stores by down-regulating the expression of the iron exporter ferroportin[9, 12]. The expression of hepcidin can be regulated at least two single ways-iron loadings and inflammatory stimulate including IL-6[13,14], IL-1[14] and lipopolysaccharide[15]. In infection cases, special the pathogen may act on macrophage, including the liver kuffer cells that would induce IL-6 produce. In turn, the IL-6 could induce the liver expression of hepcidin mRNA to change the iron metabolize. The inflammatory IL-6 pathway has also been regarded as an important pathway for the induction of hepcidin synthesis, leading to microcytic anemia with low serum iron levels observed in chronic inflammation. Nemeth et al[16] found there was a posizition in hepcidin -25 promoter to bind NF-κB. Furthermore, Peyssonnaux et al[17] figured out that TLR-4 also participated in regulation of hepcidin-25's expression.

In the clinical management of transplantation patients, the assessment of body iron status is important because a growing body of evidence suggests that iron overload has a strong negative impact on clinical outcomes[3,18]. Our study focused on these patients with evidently predisposing factor of invasive fungal disease, and found out patients with much higher serum hepcidin-25 level before transplantation were more possible involved in IFD. Moreover, after transplatation hepcidin-25 reached summit before invasive fungal infection was diagnosed by pathological proof which we could used at present. So it could help us to take prophy lactic theropy. Kanda et al[19] did not detect any adverse effect of high hepcidin levels on infection-related mortality or OS at 100 days after transplantation, although there was a marked difference in the prevalence of bacterial infection. One possible explanation for this observation is that bacterial infection of the blood was well managed by prompt and appropriate treatment with antibiotics. IFD was usually and easily sheltered but it could lead to worse prognosis. Therefore, it's necessary to predict IFD.

Conclusions

Our study revealed that the pretransplant serum hepcidin-25 level was significantly associated with IFD. Quantification of serum hepcidin-25 level could be good to predict early IFD. Larger prospective studies are warranted to confirm our findings.

Conflict of Interest

None declared.

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