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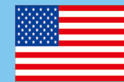

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WJH covers topics concerning experimental, clinical, oncological and transplant hematology, transfusion science, hemostasis and thrombosis, evidence-based medicine, epidemiology and nursing. Priority publication will be given to articles concerning diagnosis and treatment of hematological diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

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FRONTIER

Allogeneic stem cell transplantation in chronic myeloid leukemia patients: Single center experience

Nur Soyer, Ayse Uysal, Murat Tombuloglu, Fahri Sahin, Guray Saydam, Filiz Vural

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Abstract

Chronic myeloid leukemia (CML) is a myeloproliferative disease which leads the unregulated growth of myeloid cells in the bone marrow. It is characterized by the presence of Philadelphia chromosome. Reciprocal translocation of the *ABL* gene from chromosome 9 to 22 t (9; 22) (q34; q11.2) generate a fusion gene (*BCR-ABL*). BCR-ABL protein had constitutive tyrosine kinase activity that is a primary cause of chronic phase of CML. Tyrosine kinase inhibitors (TKIs) are now considered standard therapy for patients with CML. Even though, successful treatment with the TKIs, allogeneic stem cell transplantation (ASCT) is still an important option for the treatment of CML, especially for patients who are resistant or intolerant to at least one second generation TKI or for patients with blastic phase. Today, we know that there is no evidence for increased transplant-related toxicity and negative impact of survival with pre-transplant TKIs. However, there are some controversies about timing of ASCT, the optimal conditioning regimens and donor source. Another important issue is that BCR-ABL signaling is not necessary for survival of CML stem cell and TKIs were not effective on these cells. So, ASCT may play a role to eliminate CML stem cells. In this article, we review the diagnosis, management and treatment of CML. Later, we present our center's outcomes of ASCT for patients with CML and then, we discuss the place of ASCT in CML treatment in the TKIs era.

Key words: Chronic myeloid leukemia; Allogeneic stem cell transplantation; Tyrosine kinase inhibitors; Graft *vs* host disease; Survival

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Core tip: Tyrosine kinase inhibitors (TKIs) have changed the fatal outcomes of chronic myeloid leukemia (CML). Many studies showed that TKIs provided rapid response, few serious adverse event and impressive survival outcomes. Although, allogeneic stem cell transplantation (ASCT) is only curative treatment option for CML, since 1999, the numbers of ASCT have dropped. Currently, ASCT is offering for patients who are resistant or intolerant

to at least one second generation TKI or for patients with blastic phase. Here, we present our center's outcomes of ASCT for patients with CML and then, we discuss the place of ASCT in CML treatment in the TKIs era.

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INTRODUCTION

Chronic myeloid leukemia (CML) is a clonal myeloproliferative neoplasm that characterized by the presence of Philadelphia chromosome[1]. The incidence of CML is 1-2 cases per 100000. Reciprocal translocation of the *ABL* gene from chromosome 9 to 22 t (9; 22)(q34;q11.2) generate a fusion gene (*BCR-ABL*). BCR-ABL oncoprotein had constitutive tyrosine kinase activity that is a primary cause of chronic phase of CML[2].

Approximately 50% of patients are asymptomatic that they diagnosed incidentally after their routine laboratory tests. If they are symptomatic, symptoms are left upper quadrant pain or early satiety, fatigue, night sweats, symptoms of anemia, and bleeding due to platelet dysfunction. Splenomegaly is the main physical finding, in slightly > 50% of patients[3].

Characteristic feature of complete blood cell count is leukocytosis with basophilia and with immature granulocytes (metamyelocytes, myelocytes and promyelocytes and few myeloblasts). Thrombocytosis is frequent but severe anemia is rare[4]. Bone marrow aspirates and biopsy with conventional cytogenetics is taken from untreated patients at diagnosis. Cytogenetics must be performed by chromosome banding analysis (CBA). Fluorescence *in situ* hybridization (FISH) for t (9; 22) (q34;q11.2) and quantitative reverse transcriptase PCR (qRT-PCR) for BCR-ABL can be performed on peripheral blood[3].

The disease is classified into chronic phase (CP, most patients at presentation), accelerated phase (AP), and blast phase (BP)[4]. Clinical and hematologic criteria for the definition of AP according to World Health Organization (WHO) is the presence of one or more of the following: Persisting or increasing splenomegaly and/or white blood cells (> 10 × 10^9/L) unresponsive to therapy, 10%-19% blast cells and/or > 20% basophils in peripheral blood or bone marrow, platelet counts > 1000 × 10^9/L uncontrolled by therapy or < 100 × 10^9/L unrelated to therapy or clonal chromosome abnormalities in Ph^+ cells. Clinical and hematologic criteria for the definition of BP according to WHO is the presence of one or more of the following: Blast cells ⩾ 20% and/or extramedullary involvement excluding liver and spleen, including lymph nodes, skin, CNS, bone, and lung[4]. European LeukemiaNet (ELN) criteria for the definition of AP and BP slightly differ from WHO criteria. According to ELN, the definition of AP is the presence of one or more of the following: 15%-29% blast cells and/or > 20% basophils in peripheral blood or bone marrow, platelet counts < 100 × 10^9/L unrelated to therapy or clonal chromosome abnormalities in Ph^+ cells. The definition of BP is blast cells ⩾ 30% in peripheral blood or bone marrow and/or clonal chromosome abnormalities in Ph^+ cells[5].

The differential diagnosis of CML includes Ph^- negative chronic myeloproliferative neoplasms, leukemoid reactions, Ph-negative CML or chronic myelomonocytic leukemia.

At the diagnosis, there are several prognostic scoring systems to assess the risk of poor outcome: The Sokal score, Hasford score and the European Treatment and Outcome Study score (Table 1)[6-8]. Additionally, the stage of disease and response to tyrosine kinase inhibitor (TKI) are important factors for prognosis.

According to ELN response criteria, the complete hematologic response (CHR) is defined as white blood cell < 10 × 10^9/L, no immature granulocytes, basophils < 5%, platelet count < 450 × 10^9/L, and non-palpable spleen. The complete cytogenetic response (CCyR) is defined as no Ph (+) metaphases by CBA or < 1% BCR-ABL1-positive nuclei of at least 200 examined nuclei by FISH of peripheral blood. The partial, minor, minimal and no CyR is defined as 1%-35% Ph^+ metaphases, 36%-65% Ph^+ metaphases, 66%-95% Ph^+ metaphases and > 95% Ph^+ metaphases by CBA, respectively. Molecular response is assessed with the international scale (IS) as the ratio of BCR-ABL1 transcripts to ABL1 transcripts. Major molecular response (MMR) is defined as < 0.1% BCR-ABL1 expression. Deep molecular responses are defined as $MR^{4.0}$ (detectable disease with, 0.01% BCR-ABL1 IS or undetectable disease in cDNA with > 10000 ABL1 transcripts) and $MR^{4.5}$ (detectable disease with, 0.0032% BCR-ABL1 IS or undetectable disease in cDNA with > 32.000 ABL1 transcripts in the same volume of cDNA used to test for BCR-ABL1). Molecularly undetectable leukemia is defined as undetectable BCR-ABL with assay sensitivity ⩾ 4.5 or 5.0 logs[9].

It is recommended that either a molecular or cytogenetic test or both can be used for monitoring of CML. It's depends on local conditions of center. Routine blood counts with differentials are recommended every 1-2 wk until complete hematological response. Then, every three months, it should be evaluated to assess any side effects of TKIs. Every three months, molecular monitoring with qRT-PCR is recommended until major molecular response. Then, it can be performed every 3-6 mo. CBA of marrow cell metaphases was used for cytogenetic analysis at 3, 6 and 12 mo until CCyR. Then, it can be performed every twelve months. FISH on blood cells can be used for monitoring when the CCyR has been achieved. If patients fail to achieve therapeutic targets, progress to accelerate or blastic phase or show dysplastic changes, bone marrow biopsy and cytogenetic tests are recommended. Mutational analysis should be performed in case of progression or treatment failure[9].

Table 1 Calculation of relative risk

	Sokal score	Hasford score	EUTOS
Calculation	0.0116 × (age - 43.4) + 0.0345 × (spleen - 7.51) + 0.188 × [(platelet count/700)^2 - 0.563] + 0.0887 × (blast cells - 2.10)	0.666 when age ≥ 50 + (0.042 × spleen) + 1.0956 when platelet > 1500 × 10^9/L + (0.0584 × blast cells) + 0.20399 when basophils > 3% + (0.0413 × eosinophils) + 100	Spleen × 4 + basophils × 7
Risk definition	Exponential of the total Low risk: < 0.8 Intermediate risk: 0.8-1.2 High risk: > 1.2	Total × 1000 Low risk: ≤ 780 Intermediate risk: 781-1480 High risk: > 1480	Total Low risk: ≤ 87 High risk: > 87

Age is given in years. Spleen is given in centimeters below the costal margin (maximum distance). Blast cells, eosinophils, and basophils are given in percent of peripheral blood differential. All values must be collected before any treatment. EUTOS: European Treatment and Outcome Study.

TREATMENT OF CML

Imatinib, the first TKI, improved the 10-year survival rate from 10%-20% to 80%-90%[10]. Since its approval, two other TKIs, nilotinib and dasatinib, were approved first for second line then also for first line treatment for CML[11,12]. TKIs are now considered standard therapy for patients with chronic myelogenous leukemia.

First line treatment of CP-CML

Currently, imatinib (400 mg once daily), nilotinib (300 mg twice daily), and dasatinib (100 mg once daily) are recommended in first line therapy of CP-CML[9].

The main study of imatinib is International Randomized Study of Interferon and STI571 (IRIS). Patients with CML were randomized to receive imatinib 400 mg/d or INF-α plus low-dose subcutaneous cytarabine in this study. After a median follow-up of 19 mo, CCyR rate was 74% in imatinib arm and 9% in INF-α plus low-dose subcutaneous cytarabine (P < 0.001)[13]. In 8-year follow-up of the IRIS study, 53% of patients who treated with imatinib still had CCyR, although estimated event free survival (EFS) and overall survival (OS) rate were 81% and 93%, respectively[10].

The Dasatinib *vs* Imatinib Study in Treatment-Naive CML Patients (DASISION) and the Evaluating Nilotinib Efficacy and Safety in Clinical Trials-Newly Diagnosed Patients study (ENEST-nd) are randomized, prospective studies that showed superiority of dasatinib and nilotinib *vs* imatinib in newly diagnosed CML patients. In DASISION study, CCyR rate at 12 mo was 77% in dasatinib arm and 66% in imatinib arm (P = 0.007)[11,14]. In 3-year follow-up, responses were deeper and faster than imatinib arm. The 3-year OS and progression free survival (PFS) rates were similar both arms, but transformation to AP-CML and BP-CML was lesser than imatinib arm[15]. In ENEST-nd study, MMR rates at 12 mo were 44% in the arm of nilotinib 300 mg orally twice daily, 43% in the arm of nilotinib 400 mg orally twice daily, and 22% in the arm of imatinib (P < 0.001). The CCyR rates at 12 mo were significantly higher for nilotinib (80% for the 300-mg dose and 78% for the 400-mg dose) than for imatinib (65%) (P < 0.001)[12]. In 3-year follow-up, responses were deeper and faster than imatinib arm and transformation to AP-CML and BP-CML was lesser than imatinib arm[16].

Widespread using of TKIs is associated with drug resistance. One of the most common mechanisms of resistance involves point mutations in the kinase domain of BCR-ABL. The optimal treatment for patients failing imatinib treatment is imatinib dose escalation, a second-generation TKI or allogeneic stem cell transplantation (ASCT)[1]. Recently, there are some experimental studies using combination of TKIs to overcome the drug resistance[17-19]. They reported that combination of TKIs could overcome and prevent resistance. Combined TKIs approach should be investigated in further clinical trials in the subset of patients with TKI resistance.

Patients should be followed up according to definition of ELN response criteria (Table 2). If patients do not achieve a CHR by 3 mo, switching to a second TKI should be considered. If patients had > 10% BCR-ABL1 transcript level at 3 mo, it is recommended that serial molecular monitoring should be performed for 3 mo. If patients had > 10% BCR-ABL1 transcript level at 6 mo, therapy should be changed. If patients do not achieve CCyR by 12 mo, it requires a change in therapy. At any time, therapy should be changed, if patients loss of CHR or CCyR or PCyR or confirmed loss of MMR or determined new mutations and/or CCA/Ph+[9].

Second line treatment of CP-CML

For patients who had intolerance to first line TKI, anyone of the other TKIs approved first line therapy can be used. Patient's comorbidities and toxicity profile of TKIs are considered in the choice of therapy[9].

For patients who had failure of TKI in first line, other TKIs approved first line therapy that patient did not use, bosutinib or ponatinib were recommended. Bosutinib was studied in patients that were resistant to or intolerant of imatinib. The CHR and CCyR rates were 86% and 41%, respectively. The 2-year PFS and OS rates were 79% and 92%, respectively[20]. Ponatinib is the only TKI with activity in patients with the T315I mutation. In phase Ⅱ Ponatinib Ph ALL and CML Evaluation study, among 267 patients with chronic-phase CML, 56% had a major cytogenetic response, 46% had a complete cytogenetic response, and 34% had a major molecular response[21]. So, bosutinib (500 mg once daily) and ponatinib (45 mg once daily) have been approved for patients resistant to prior therapy.

Table 2 European LeukemiaNet response criteria to tyrosine kinase inhibitors at first line

	Optimal	Warning	Failure
Baseline	NA	High risk or CCA/Ph+, major route	NA
3 mo	BCR-ABL1 ≤ 10% and/or Ph+ ≤ 35%	BCR-ABL1 > 10% and/or Ph+ 35%-95%	Non-CHR and/or Ph+ > 95%
6 mo	BCR-ABL1 ≤ 1% and/or Ph+ 0	BCR-ABL1 1%-10% and/or Ph+ 1%-35%	BCR-ABL1 > 10% and/or Ph+ > 35%
12 mo	BCR-ABL1 ≤ 0.1%	BCR-ABL1 > 0.1%-1%	BCR-ABL1 > 1% and/or Ph+ > 0
Then, and any at time	BCR-ABL1 ≤ 0.1%	CCA/Ph- (-7 or 7q-)	Loss of CHR Loss of CCyR Confirmed loss of MMR mutations CCA/Ph+

In 2 consecutive tests, of which one with a BCR-ABL1 transcripts level ≥ 1%. This table was originally published in Baccarani *et al*[5]. CCA/Ph+: Clonal cytogenetic abnormalities in Ph-positive cells; CCA/Ph-: Clonal cytogenetic abnormalities in Ph-negative cells; Ph: Philadelphia chromosome; CCyR: Complete cytogenetic response; MMR: Major molecular response; NA: Not applicable.

Treatment of AP-CML

The therapeutic approach in AP-CML differs according to whether the patient is TKI naive or has progressed from CP while taking a TKI. All recommendations are based on results of single-arm, retrospective and prospective studies. For TKI naive patients; it is recommended a TKI (imatinib 400 mg twice daily or dasatinib 70 mg twice daily or 140 mg once daily). Allogeneic donor search should be done. ASCT is recommended for the AP patients who do not achieve an optimal response with TKI[9]. Response rate was reported higher with second generation TKIs than imatinib[22].

For patients who progressed from CP to AP-CML while taking a TKI; it is recommended anyone of the TKIs that were not used before progression. Allogeneic donor search and ASCT should be performed all patients[9].

Treatment of BP-CML

It is recommended combinations of induction chemotherapy and TKIs for patients with BP-CML. ASCT is recommended for all BP-CML patients who are eligible [9].

ASCT

ASCT is a highly effective treatment for CML. Since TKIs were used routinely in first line treatment and were safe and highly effective at controlling CP-CML, the numbers of allografts performed for CML have dramatically decreased[23]. Although outcomes of ASCT improved over years, HSCT is still limited to patients with an available donor and remains associated with significant morbidity and mortality[24]. However, ASCT remains an important therapeutic option for CML, especially for patients who are resistant or intolerant to at least one second generation TKI or for patients with blastic phase[9]. Another issue is keep in mind that BCR-ABL signaling is not necessary for survival of CML stem cell and TKIs were not effective on these cells[25,26]. ASCT is still had the potential for cure.

In this report, we present our single center experience of the outcomes of ASCT for patients with CML. Then, we will review our data with the literature of ASCT for CML.

CASE SERIES

Ten patients (3 female and 7 male) with CML were treated with ASCT in our center between October 2000 and December 2015. The median age at the transplantation was 50 (range 22-65) years. All patients were in chronic phase at diagnosis. Only one patient did not receive imatinib, this patient treated with interferon and hydroxyurea. Others received at least imatinib. One patient had primary imatinib resistance and 8 had lost their response. Four patient who had lost their response to imatinib received second-line TKIs. At the time of transplantation, 5 of all were in first CP, 3 were in 2nd CP and 2 was in AP. Time from diagnosis to ASCT was 61.5 (range 14-133) mo. Nine of all transplantation were matched sibling donor and one was an antigen mismatched (HLA A antigen) unrelated donor transplantation. Patient's characteristics are shown in Table 3.

Seven patients received myeloablative conditioning regimens (busulfan 3.2 mg/kg per day 4 d and cyclophosphamide 60 mg/kg per day 2 d) and 3 patients were received non-myeloablative regimens (fludarabine 30 mg/m^2 per day 5 d and busulfan 3.2 mg/kg per day 2 d; fludarabine 30 mg/m^2 per day 5 d, busulfan 3.2 mg/kg per day 2 d and, cyclophosphamide 350 mg/m^2 per day 3 d). Cyclosporine (2 mg/kg per day day -1, levels maintained at 200-300 μg/L until dose reduction) and methotrexate (15 mg/m^2 on day +1, 10 mg/m^2 on day +3, +6, +11 for myeloablative regimens and 10 mg/m^2 on day +1, +3 and +6 for non-myeloablative regimens) were used for graft *vs* host disease (GVHD) prophylaxis. In all patients, peripheral blood stem cell grafts were used.

All patients were engrafted. The median neutrophil and platelet engraftment times were 13 (10-25) d and 14.5 (10-30) d, respectively (Table 4). The median follow-up was 16.5 (3-117) mo. Only 3 patients are still alive without disease. The median follow-up of these patients were 87 (50-117) mo. Five patients died of complications after ASCT including acute GVHD (n = 3), and infection (n = 2). Two of all patients relapsed at 19 (molecular relapse) and 6 (hematological relapse) months from the date of ASCT.

Although our cohort is small, most of patients achieved molecular remission after transplantation. Only 2 patients died because of blastic crises and granulocytic sarcoma.

Table 3 Patients characteristics

Patient	Sex	Age at ASCT (yr)	Disease phase at ASCT	Time from diagnosis to ASCT (mo)	Indication for ASCT	Donor sex
1	M	22	1st AP	50	Resistance to imatinib and clonal evolution	M
2	F	51	2nd CP	14	Previous myeloid blastic phase	M
3	M	25	2nd CP	61	Previous lymphoid blastic phase	M
4	M	33	1st CP	37	Resistance to imatinib and dasatinib	M
5	F	54	1st AP	103	Resistance to imatinib, nilotinib and dasatinib 1st accelerated phase	F
6	M	65	1st CP	133	Resistance to imatinib and nilotinib	F
7	F	49	1st CP	62	Resistance to imatinib	M
8	M	53	2nd CP	63	Previous myeloid blastic phase	M
9	M	41	1st CP	51	Resistance to imatinib	M
10	M	61	1st CP	75	Resistance to imatinib, nilotinib and dasatinib	F

ASCT: Allogeneic stem cell transplantation; AP: Accelerated phase; CP: Chronic phase; M: Male; F: Female.

Others died because of acute GVHD and infection. However, only 3 patients achieved long term survival, ASCT has a place for treatment of CML.

DISCUSSION

As we mentioned above, ASCT is still important therapy for CML patients. In 1982, different groups were reported ASCTs with bone marrow graft from HLA-matched siblings[27-29]. Then, it was shown that CML patients who received T-cell depleted transplants with or without GVHD had higher probabilities of relapse than recipients of non-T-cell depleted allografts without GVHD. These data support graft-*vs*-leukemia (GVL) effect independent of GVHD[30]. Other reports showed that donor leukocyte infusions (DLI) for treatment of recurrent CML after ASCT could achieve stable remissions[31-33].

Outcomes of ASCT for CP-CML patients continued to improve with general improvements in transplant management and powerful GVL effect of DLI. In the post-TKIs era, there are some reports evaluating outcomes of ASCT and potential negative effect of TKIs[34-38].

According to European Society for Blood and Marrow Transplantation (EBMT) data, the 2-year OS, transplantation-related mortality (TRM) and relapse rate in patients transplanted between 2000 and 2003 were 61%, 30% and 22%, respectively[34]. Eighty-four patients with CML who underwent ASCT were evaluated in 3 groups according to the reason of ASCT: Group Ⅰ (early transplantation in low-risk patients, EBMT scores 0-1), group Ⅱ (imatinib failure in first CP), and group Ⅲ (advanced disease). At a median follow-up of 30 mo, the 3-year OS was 88% in group I, 94% in group Ⅱ and 59% in group Ⅲ. TRM was 8%[35]. In a cohort study, it was compared the outcomes of imatinib *vs* ASCT for AP-CML. In ASCT arm, median follow-up was 51 mo, and the 6-year OS, EFS, and PFS were 83.3%, 71.8% and 95.2%, respectively. In imatinib arm, median follow-up was 32 mo, and the 6 year OS, EFS and PFS were 51.4%, 39.2% and 48.3%, respectively. Patients treated with ASCT were significantly higher OS (P = 0.023), EFS (P = 0.008) and PFS (P = 0.000) than patients treated with imatinib[36]. Pfirrmann *et al*[37] compared two consecutive German studies Ⅲ (recruitment from 1995 to 2001) and ⅢA (recruitment from 1997 to 2004) on chronic myeloid leukemia. They reported that HLA matching, age of transplantation ≤ 44 and time from diagnosis to ASCT ≤ 1 year had a significant association with improved survival. They also reported that improvement of transplantation practice over years was associated with better survival. These findings suggested that the timing of ASCT is an important factor on survival outcomes.

According to Center for International Bone Marrow Transplantation Research (CIBMTR) data in TKI era, 3-year OS and disease free survival (DFS) rates were 36% and 27% in second CP, 43% and 37% in AP, and 14% and 10% in BP. Pre-transplant imatinib had no association with transplant outcomes, including acute and chronic GVHD[38]. In a study with CIBMTR data, they reported that pre-transplant imatinib therapy was associated with improved survival after transplantation and, they showed similar acute GVHD rates both using and not using imatinib before transplantation[39]. Fifty-one patients with CML underwent ASCT for advanced disease at diagnosis or for treatment failure with TKIs. At a median follow-up of 71.9 mo, the 8-year OS, EFS, relapse, and non-relapse mortality (NRM) were 68%, 46%, 41% and 23%, respectively[40]. Another study demonstrated that OS, DFS, relapse and NRM rates were similar between pre-transplant imatinib arm and no imatinib arm. On the other hand, mortality was higher in CP patients with suboptimal response than in CP patients with CCyR or major CyR on imatinib[41]. In a retrospective study, it was demonstrated that there was no evidence for increased transplant-related toxicity with pre-transplant dasatinib and nilotinib therapy[42]. In a small study, they showed that using dasatinib and nilotinib before ASCT did not increase transplant-related toxicity or GVHD[43]. According to these data, there is no evidence for increased transplant-related toxicity and negative impact of survival with pre-transplant TKI.

Goldman *et al*[44] reported that 15-year OS and relapse rates were 88% and 8% for sibling donor ASCT and 87% and 2% for unrelated donor ASCT, respectively. Recent randomized, prospective study evaluated differences between early allogeneic HSCT (group A) and best drug

Table 4 Transplantation outcomes

Patient	PNL engraftment (d)	PLT engraftment (d)	Acute GVHD	Chronic GVHD	Post-transplant disease status	Last status
1	12	18	No	No	Molecular relapse and granulocytic sarcoma	Died
2	15	16	No	No	Blastic crises	Died
3	18	30	No	No	Remission	Died
4	14	14	Yes	No	Remission	Died
5	25	21	Yes	Yes	Remission	Alive
6	19	15	Yes	No	Remission	Died
7	10	10	No	No	Remission	Alive
8	10	10	Yes	No	Remission	Died
9	11	14	Yes	No	Remission	Alive
10	12	13	Yes	No	Remission	Died

GVHD: Graft *vs* host disease; PNL: Neutrophil; PLT: Platelet.

treatment (group B) in patients eligible for both strategies. The 10-year OS was not different between group A (76%) and group B (69%). Patients of group A with low risk EBMT score (10-year OS 85%) had significantly higher survival (median $P < 0.001$) compared with patients with high-risk (10-year OS 41%) and non-high-risk Euro score in group B (median $P = 0.047$; 10-year OS 73%)[45]. The studies demonstrated that ASCT is still an option for selected CML patients.

Myeloablative vs non-myeloablative regimens

The curative effect of ASCT in CML is largely associated with the immune effect (GVL) mediated by alloreactive donor T cells. Reduced intensity conditioning (RIC) regimens provided reduced toxicity and rapid engraftment for elderly patients or those with comorbidities. In a small study was demonstrated that GVL effect may be insufficient and cytoreduction is required to provide cure with ASCT for CML[46]. The 5-year OS and DFS was 85% ± 8% with fludarabine, low-dose busulfan, and anti-T-lymphocyte globulin containing non-myeloablative (NMA) regimen[47]. In a study which was evaluated ASCT with RIC regimen for CML, after a median follow-up of 30 mo, 35.3% of patients were still alive[48]. Kebriaei *et al*[49] evaluated outcomes of 64 CML patients with advanced-phase disease who were treated with fludarabine-based RIC regimens. The 5-year OS and PFS were 33% and 20%, respectively. TRM was 33% at 100 d and 48% at 5 years after ASCT. In a study that were compared outcomes of MA conditioning regimen (56 patients) *vs* RIC regimen (28 patients), the 5- and 10-year leukemia-free survival and OS were similar. On the other hand, relapse rate was higher in patients receiving RIC regimen, whereas mortality rate was higher in patients receiving MA regimen[50]. In a large multicenter CIBMTR analysis compared RIC regimens with NMA regimens, relapse risk was lower and DFS was higher with RIC regimens than NMA regimens[51]. According to all these data, RIC regimens have had a place for elderly patients and patients who had comorbidities, but NMA regimens were inferior to RIC regimens.

In a study that was used data from the CIBMTR, they compared outcomes in patients who treated with ASCT following MA conditioning with cyclophosphamide (Cy) in combination with TBI, oral busulfan (Bu) or intravenous (IV) busulfan[52]. They concluded that Cy in combination with IV Bu was associated with less relapse than TBI or oral Bu. NRM and OS were similar.

GVHD prophylaxis

Another important issue is GVHD prophylaxis for ASCT. In CML, GVHD prophylaxis can influence the outcomes. T-cell depletion was associated with higher relapse rate, but DLIs were controlled the disease relapse in CML[30-33]. Zuckerman *et al*[53] evaluated 38 patients who treated with ASCT using partial T cell depletion (TCD) and preemptive DLI, without post-transplant GVHD prophylaxis. The 5-year LFS and OS were 78.95% and 84.2%, respectively. Acute GVHD rate was 18% in post-transplant patients and 24% in patients receiving DLI. They concluded that partial TCD and preemptive DLI was reduced the GVHD risk. In a small study, the CCyR was induced in 8 of 9 CML patients who treated with ASCT using an alemtuzumab-based RIC regimen. GVHD incidence was low but disease relapse was frequent[54].

Post-transplantation relapse

DLIs, TKIs, chemotherapy and second ASCT can be used for treatment of relapse CML after ASCT[55-59]. Olavarria *et al*[55] reported response to imatinib in 128 patients with CML relapsing after ASCT. The CCyR rate was 58% for patients in CP, 48% for AP and 22% for patients in BC. The CyR rates were 63% for CP or AP and 43% for BP in a small study that evaluated response to imatinib in 28 relapse CML patients after ASCT[58]. They concluded that imatinib is highly effective treatment for relapse CML after ASCT. A retrospective study was evaluated pre-DLI factors associated with prolonged survival in remission without secondary GVHD. They reported that approximately 50% of responding patients treated with DLI had GVL effect without secondary GVHD. Prolonged survival in remission without secondary GVHD was observed in patients who were given DLI beyond 1 year from ASCT for molecular and/or cytogenetic relapse[60].

Donor source

Transplantation from HLA-matched sibling donor (MSD) has been associated with the most favorable outcomes[61-64].

In a study from China, they compared the long-term outcomes of HLA-MSD with mismatched related donor (MRD) and unrelated donor (URD) ASCT for CML. They concluded that OS is similar between HLA allele-matched URD and MSD transplantation, but OS is lower in MRD and mismatched URD transplantation than MSD transplantation[64].

Although MSD transplantation has favorable outcomes, MSD is available for only one third of the patients. So, we can choice MRD, URD or haploidentical donor for ASCT. Previously, haploidentical donor transplantation has had inferior outcomes than MSD. These results are related to higher GVHD and TRM rates. Post-transplantation cyclophosphamide improved the outcomes of haploidentical transplantation. Ma *et al*[65] compared outcomes of 67 haploidentical ASCT and 23 MRD ASCT for patients with BP-CML or CP-CML from blast crisis. The 3-year OS and RFS rates were 60.0% and 51.1 for haploidentical transplantation and 55.3% and 47.8% for MRD transplantation, respectively. They concluded that haploidentical transplantation is an option for BP-CML with comparable survival to MRD transplantation.

Transplantation time

There are some reports that time from diagnosis to ASCT less than 12 mo is associated with better outcomes for patients advanced phase CML[36,37]. Xu *et al*[66] reported that for T315I mutation positive CML patients, haploidentical ASCT is highly curative treatment and immediate ASCT could result in promising survival for patients in CP/AP. There are different suggestions about patients in CP CML who failed second-line TKI. These patients could receive a third-line agent or be considered for SCT. Patients in BP should receive intensive chemotherapy with or without a TKI. If patients achieved second chronic phase, ASCT should be considered[9].

CONCLUSION

ASCT is still an important option for treatment of CML. There are some questions about timing of transplantation, optimal conditioning regimen and optimal GVHD prophylaxis. Some reports indicate that using TKI before ASCT is not associated with inferior ASCT outcomes. Non-myeloablative ASCT seems to be feasible for older and medically infirm patients. Relapse after ASCT can be manage with DLIs and TKIs.

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MINIREVIEWS

Current approach to disseminated intravascular coagulation related to sepsis - organ failure type

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Abstract

Disseminated intravascular coagulation (DIC) is a syndrome characterized by the systemic activation of blood clotting, which generates large amount of intravascular thrombin and fibrin. Various diseases may cause acceleration of the clotting cascade, inactivate the endogenous anticoagulants and modify fibrinolysis, having thus the formation of micro thrombi in the systemic circulation. The abnormalities in the hemostatic system in patients with DIC result from the sum of pathways that generate both hypercoagulability and augmented fibrinolysis. When the hypercoagulability state prevails, the main manifestation is organic failure. This subtype of DIC is often referred as "organ impairment" type, frequently seen in patients suffering from severe sepsis. To identify the underlying infection, early initiation of culture-based antimicrobial treatment, and to resolve any infection source promptly are keystone actions of DIC related to sepsis prevention and treatment. These should be combined with specific treatment related to each DIC subtype. In the context of septic shock, DIC is associated to increased severity, greater number and seriousness of organ failures, more frequent side-effects from treatment itself, and worse outcomes. Therefore, we ought to review the information available in the literature about approach and management of DIC in severe sepsis.

Key words: Septic shock; Disseminated intravascular coagulation; Coagulation impairment; Organ failure; Antithrombin; Sepsis

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Core tip: Disseminated intravascular coagulation (DIC) is a syndrome characterized by the systemic activation of blood clotting, which generates large amount of intravascular thrombin and fibrin. In the context of severe sepsis and septic shock, DIC is related to increased severity, greater number and seriousness of organ failures, more frequent side-effects from treatment itself, and worse outcomes. We ought to review the most important and updated information available in the literature about DIC in severe sepsis and septic shock.

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INTRODUCTION

Disseminated intravascular coagulation (DIC) is a syndrome characterized by the systemic activation of blood clotting that generates a large amount of intravascular thrombin and fibrin. This process results in small and medium vessel thrombosis and, eventually, organ failure and severe hemorrhage[1,2]. DIC could be the consequence of infections, hematologic malignancy, obstetric complications, trauma, aneurisms or hepatopathy. Each etiology signifies individual hazards related to the underlying disorder. Therefore, the diagnosis and treatment should be dictated by the disease[3,4].

In the context of septic shock, DIC is related to increased severity, number and seriousness of organ failures, more frequent side-effects from treatment itself, and worse outcomes, including death[5,6]. Therefore, we ought to review the most important and updated information available in the literature about DIC in severe sepsis and septic shock setting.

NORMAL HEMOSTASIS

Hemostasis is an organized process that aids to maintain vascular integrity. In the presence of endovascular damage, thrombin generation with simultaneous negative feedbacks and coordination of fibrinolysis occur, to avoid massive hemorrhage or excessive thrombosis. The first step in hemostasis is the formation of a platelet plug over the damaged zone[7]. On the surface of platelets, Integrins interact with each other and with endothelial cells surface through the von Willebrand factor and fibrinogen. Nevertheless, the formation of a platelet plug is not enough to achieve stable hemostasis, given that the contribution of a fibrin mesh to stabilize the structure of the clot is needed.

Clotting cascade

Physiologic clotting initiates with tissue factor (TF) and activated Factor Ⅶ (FⅦ) complexes that cleave Factor Ⅹ (FⅩ) into activated FX. This initial step has a short duration, due to quick inhibition of TF-aFⅦ complexes by the tissue factor inhibitor. The second pathway starts with Factor Ⅸ (FⅨ) that cracks into activated FⅨand joins activated FⅧ to transform FⅩ into activated FⅩ. Activated FⅩ forms a complex with activated Factor Ⅴ (FⅤ), with both phospholipids on platelet surface and calcium to turn prothrombin into thrombin[8]. Subsequently, thrombin turns fibrinogen into fibrin. At that time, activated Factor XIII (FXIII) forms crossbred fibrin connections inside the clot, which serve as an additional support. Finally, fibrin clots are degraded by a protease called plasmin (Figure 1)[8].

DIC PATHOPHYSIOLOGY

Any alteration in hemostasis balance could generate hemorrhage or thrombosis[8]. In critically ill patients, this alteration is usually associated with sepsis, malignancy, and multiple trauma. These diseases usually accelerate the clotting cascade, inactivate endogenous anticoagulants, and modify fibrinolysis, resulting in micro thrombi formation in the systemic circulation[3].

The abnormalities in the hemostatic system in patients with DIC result from either hypercoagulability or hyperfibrinolysis[8] (Figure 2). When hypercoagulability prevails, the main clinical manifestation is organ failure. This type of DIC is referred as organ impairment type (both hypercoagulability and/or hypo-fibrinolysis exist)[9]. Organ impairment or organ failure DIC subtype is often seen in patients with severe sepsis. The activation of the coagulation cascade is an important part of the defense mechanisms to prevent infection dissemination. The increase in serum plasminogen activator inhibitor type 1 (PAI-1) caused by high levels of cytokines and lipopolysaccharides (LPS) in the blood of septic patients has been identified as one of the causes of hypofibrinolysis. Moreover, activated neutrophils in patients with sepsis liberate histones, neutrophil elastase and Catepsin G as a defense mechanism against pathogens[10]. Histones promote endothelial cell apoptosis, and platelet aggregation; meanwhile, neutrophil elastase inhibit Antithrombin (AT) and the Catepsin G decrease levels of the tissue factor pathway inhibitor (TFPI) promoting thrombus formation[10,11].

Cytokines

Endotoxin LPS are a component of the external membrane of gram negative bacteria, responsible of many of the cases of sepsis[12]. The entrance of endotoxin into systemic circulation causes the production of pro inflammatory cytokines. The consequent tissue damage is aggravated through free radicals generated by activated leucocytes. This causes an imbalance in normal hemostasis with the ulterior formation of thrombi in small and medium blood vessels that promote loss of vascular tone. All of this mechanisms contribute to the development of multiple organ failure[11-13].

Tumor necrosis factor: Tumor necrosis factor alpha (TNF-α) is synthesized in macrophages, and it is amongst the first cytokines to appear when endotoxin reaches blood circulation. It grasps it maximum concentration at 90 min from stimuli; then, it gradually disappears despite if the toxic stimulus remains. TNF-α has an important role initiating the inflammatory cytokine cascade and tissue damage. It has effects over monocytes, neutrophils, and vascular endothelium causing the production of other pro inflammatory interleukins (1b, 6 or 8). Furthermore, it stimulates the production of adhesion molecules such as

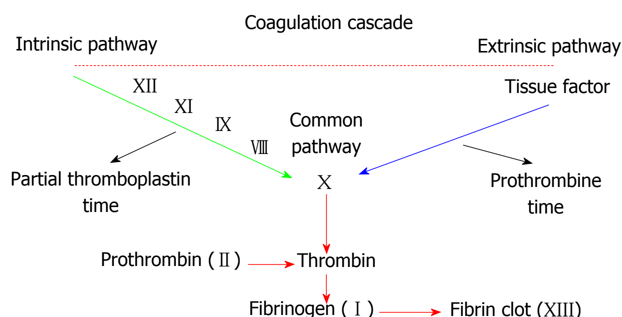

Figure 1 Schematic drawing of the coagulation cascade.

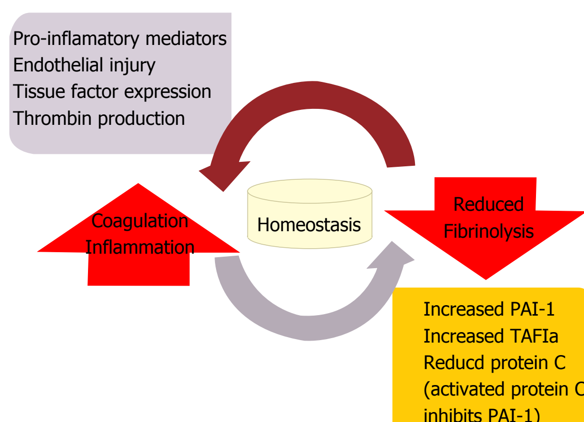

Figure 2 Mechanisms associated with hypercoagulability and/or hypofibrinolysis observed in sepsis related disseminated intravascular coagulation. PAI-1: Plasminogen activator inhibitor type 1; TAFIa: Thrombin activatable fibrinolysis inhibitor.

Intercellular Adhesion Molecule-1, vascular cell adhesion molecule-1 or E-Selectin.

Interleukin 1b: When the LPS enter the bloodstream, one can detect interleukin 1b (IL-1b) in plasma, and its presence serves as a severity marker. Patients with septic shock have high levels of IL-1b. It has been shown that the administration of this protein in primates induces a reduced fibrinolytic response equivalent to the one obtained with LPS or TNF-α. This suggests that IL-1b contributes to hypo-fibrinolysis mediated by PAI-1 in the presence of endo-toxemia[14,15].

IL-6: Endothelial cells synthesize IL-6 in presence of LPS. It also appears in the general circulation just after TNA-α shows up. IL-6 has a pathophysiologic role during sepsis as a clotting activator, and its concentration correlates with the disease severity[14,15].

Other cytokines: Other molecules participate in the inflammatory process in presence of the LPS: IL-12, IL-8, and interferon-γ. Nevertheless, their role in DIC is not yet well defined[15].

DIC DIAGNOSIS

At the bedside, is necessary to consider the clinical conditions that could alter the commonly used laboratory tests to diagnose DIC. Ergo, the diagnosis requires clinical expertise along biochemical workshop. The recurrently used test that might be affected include platelet count, prothrombin time (PT), fibrinogen, and fibrin degradation products (FDP), among others. Some clinical guidelines issued recommendations regarding this aspect[1,16,17]. In 2013 the International Society of Thrombosis and Hemostasis published recommendations for diagnosis and treatment of disseminated intravascular coagulation[18]. This guidance was based on a previous consensus by the British Committee for Standards in Hematology, the Japanese Society of Thrombosis and Hemostasis, and the Italian Society for Hemostasis and Thrombosis (Società Italiana per lo Studio dell'Emostasi e della Trombosi - SISET). They stated that in sepsis related DIC the major variation is either hyper-coagulation or hypo-fibrinolysis. As mentioned above, the main clinical manifestation is organ failure, so several validated score systems to recognize DIC have been distributed using platelet count, prothrombin time, and anti-thrombin. The Japanese Association of Acute Medicine (JAAM) published a score system to detect sepsis related DIC, with a sensitivity and specificity of 100% and 65.0% respectively[5,19,20] (Table 1). Recently, Iba *et al*[21] proposed a modified version of the JAAM-DIC diagnostic criteria. They suggest to replace Systemic Inflammatory Response Syndrome (SIRS) by antithrombin activity, since SIRS is no longer used for the diagnosis of Sepsis. The new criteria could diagnose the same number of patients with comparable severity (mortality, 34.6% *vs* 34.8%). Also, mortality increased as the baseline antithrombin activity decreased (patients with a baseline antithrombin activity ≥ 70% had a mortality of 26.5% *vs* 35.5% for those with an antithrombin activity < 70%). Despite this promising results, future studies to examine the worth of the modified scoring system in different populations are warranted[21].

Laboratory findings

A complete coagulation examination, including prothrombin time and platelet count is essential[4]. In some types of DIC (bleeding, massive hemorrhage, and asymptomatic) identifying the elevation of fibrin-associated biomarkers (D dimer, FDP, and soluble Fibrin) is useful to establish diagnosis[9]. Table 2 highlights the laboratory tests useful to diagnose DIC in a septic patient. It is important to consider that a coagulation disorder has around 35%-40% chance to be related to any other cause beside sepsis. A positive result does not guarantee the diagnosis. Delabranche *et al*[22] in 2016 published a multicenter, prospective observational study completed in 4 intensive care units in France. They used de JAAM score, sequential organ failure assessment score, and the acute physiology and chronic health evaluation II to identify patients with DIC at early stage. They concluded that a combination of PT, endothelium-derived

Table 1 Score for disseminated intravascular coagulation diagnosis established by the Japanese Association of Acute Medicine

Parameter	Points
SIRS criteria	
3 or more	1
2-0	0
Platelet count (× $10^3/\mu L$)	
< 80 or a reduction of > 50% in 24 h	3
80-120 or a reduction of > 30% in 24 h	1
> 120	0
Prothrombin time	
1.2 times over control or higher	1
< 1.2 times over control	0
Fibrin degradation products/fibrinogen (mg/L)	
25 or more	3
10 to 24	1
< 10	0
Diagnosis DIC: 4 or more points	

SIRS: Systemic inflammatory response syndrome; DIC: Disseminated intravascular coagulopathy.

Table 2 Laboratory findings in sepsis-related disseminated intravascular coagulation

Test	Alteration	Other causes
Platelet count	Reduction	Bone marrow abnormalities
Anti-thrombin/C protein	Reduction	Hepatic failure, capillary leakage syndrome
Prothrombin time	Extended	Hepatic failure, vitamin K deficiency
Soluble fibrin/thrombin	Increased	VTD, surgery
vWF-PP/PAI-1	Increased	Organic failure
aPTT	Bifasic wave	Infection
ADAMTS-13	Reduction	Hepatic failure, thrombotic microangiopathy
FDP/DD	Increased	VTD, surgery

VTD: Venous thromboembolic disease; vWF-PP: Von Willebrand factor pro-peptide; PAI-1: Type 1 plasminogen activator inhibitor; ADAMTS-13: A desintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; FDP: Fibrin degradation products; DD: D-dimer; aPTT: Activated partial thromboplastin time.

$CD105^+$-microparticles, and platelet count at admission could predict the absence of disseminated intravascular coagulation[22].

Liu *et al*[23] found four thrombin derived biomarkers that were triggered before PT, activated partial thromboplastin time (aPTT), or platelet count became altered. These markers include fibrinopeptide type A, soluble fibrin monomer complex, prothrombin fragment 1 + 2 (F1 + 2), and the thrombin-antithrombin complex. The F1 + 2 represents the total amount of fibrin produced, while the other three markers only show it partially. F1 + 2 is considered the most sensitive marker of thrombin production.

In the last few years the identification of endothelial damage markers and inflammatory cascade activators have made possible to find coincidences between the inflammation trigger mechanisms and coagulation. This extend the possibilities for future treatment targets[11].

Table 3 Treatment recommendations amongst different types of disseminated intravascular coagulation

Dysfunction	Recommended treatment
Pre-DIC	Treat cause and UFH 70 IU/kg per day or LWMH anti-Xa target: 0.8-1.2
Multiple organ failure	Treat cause and AT 30 IU/kg per day of 3 d
Hemorrhagic	Treat cause and Hemo-transfusion Anti-fibrinolytics Protease synthetic inhibitor
Massive hemorrhage	Treat cause and Hemo-transfusion Anti-fibrinolytics Protease synthetic inhibitor

DIC: Disseminated intravascular coagulopathy; UFH: Unfractionated heparin; LWMH: Low molecular weight heparin; Xa: Activated X factor; AT: Anti-thrombin.

DIC TREATMENT

To identify the underlying infection, early initiation of culture-based antimicrobial treatment, and to resolve any infection source promptly are keystone actions of DIC related to sepsis prevention and treatment. Table 3 lists key recommendations for the treatment of different types of CID.

The Surviving Sepsis Campaign guidelines[24], do not recommend treatment of any associated coagulopathy as for the lack of evidence to support it. Recently, Umemura *et al*[25] reported a meta-analysis of anticoagulation therapy in three different types of patients: (1) septic patients without coagulopathy; (2) patients with sepsis induced coagulopathy; and (3) patients with induced sepsis DIC. They identified that only septic induced DIC patients had a reduced mortality with no difference in the prevalence of hemorrhagic complications[25]. In septic patients, biomarkers of the homeostasis loss, such as histones (H3, H4), the TFPI, and the neutrophil extracellular traps are useful to determine whether to start treatment[26].

Antithrombin

AT has proven to be effective to revert sepsis induced DIC. As mentioned above, when germs disseminate throughout the organism, a diffuse coagulopathy that results in massive thrombi formation in small and medium blood vessels occur[13]. The KybertSept trial[27] was the first to evaluate the effectiveness of AT substitution in patients with severe sepsis and septic shock. The results showed an increase in the incidence of bleeding complications related to AT use. It is important to reflect that some of their patients used heparin as deep vein thrombosis prophylaxis. A sub-analysis of patients without heparin prophylaxis showed a reduction of adverse effects in AT group[27]. Later on, Gando *et al*[28] showed that in patients

with activated-AT levels of 50%-80%, the administration of AT at a dose of 30 UI/kg perday during 3 d improved platelet counts, and reduced the score punctuation for sepsis associated DIC without increasing bleeding events[28].

Heparin use

Antithrombin-Ⅲ (AT-Ⅲ) inactivates thrombin and other proteases, including FXa[29]. Heparin attaches to a AT-Ⅲ producing a conformational change that increases AT-Ⅲ activity. The unfractionated heparin (UFH) dose in Pre-DIC is 70 UI/kg per hour in continuous infusion for 5-7 d[23]. There are few randomized controlled trials evaluating the utility of heparin in DIC. Liu *et al*[23] shown that low molecular weight heparin was superior to UFH due to a higher inhibition of FXa[29]. The utility of other compounds like Fondaparinux and Danaparoid sodium is restricted to asymptomatic DIC for risk reduction of thrombotic events[9].

Blood components administration

Because of coagulation factors (specially fibrinogen) and platelet consumption, most clinical guidelines[1,16,17] recommend blood components administration only in hemorrhagic and massive hemorrhage DIC. The recommended platelet goal count has been established at 50 × 10^3/μL if active bleeding or 20 × 10^3/μL along high risk of hemorrhage. If PT or aPTT are 1.5 times over the standard, or fibrinogen is below 1.5 g/dL, fresh frozen plasma (15 mL/kg) is indicated. If volume restriction is intended, a concentrate of prothrombin complex, cryoprecipitates, or purify fibrinogen concentrates are preferred[1,16,17].

Human recombinant thrombomodulin

Thrombomodulin may reduce massive thrombotic events caused by the expression of extracellular histones observed in sepsis DIC[26]. In the double blind controlled study, Vincent *et al*[30] administered human recombinant thrombomodulin to patients with sepsis induced DIC that developed one or more organ failures and an international normalized ratio > 1.4. The dose of 0.06 mg/kg per day for 6 d along with conventional treatment reduced the severity of hematologic failure and reduced DIC incidence. Further trials are needed to safely recommend the therapy.

CONCLUSION

In critically ill patients, the early diagnosis of coagulopathy is essential to reduce morbidity and mortality. Identification of sepsis related DIC is difficult, especially when precise laboratory tests are not available. Clinicians should suspect the diagnosis in every severe sepsis or septic shock patient, and use whatever tools accessible to investigate it. It is important to treat promptly even subtle changes linked to coagulopathy, to diminish the extent of DIC.

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ORIGINAL ARTICLE

Basic Study

Intestinal heme absorption in hemochromatosis gene knock-out mice

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Abstract

AIM
To investigat the influence of hemochromatosis gene (Hfe) mutation on ^{59}Fe labelled duodenal heme absorption in mice.

METHODS
Heme absorption was measured in Hfe wild type and $Hfe^{(-/-)}$ mice by the duodenal tied loop and by oral gavage methods. The mRNA expression of heme oxygenase (*HO-1*), *Abcg2* and *Flvcr1* genes and levels were determined by quantitative polymerase chain reaction.

RESULTS
Heme absorption was significantly increased in homozygous $Hfe^{(-/-)}$ mice despite significant hepatic and splenic iron overload. While duodenal *HO-1* mRNA was highly expressed in the wild type and $Hfe^{(-/-)}$ heme-treated group following 24 h heme administration, *Flvcr1a* mRNA decreased. However, *Abcg2* mRNA expression levels in duodenum remained unchanged.

CONCLUSION
Heme absorption was enhanced in $Hfe^{(-/-)}$ mice from

both duodenal tied-loop segments and by oral gavage methods. *HO-1* mRNA levels were enhanced in mice duodenum after 24 h of heme feeding and may account for enhanced heme absorption in Hfe$^{(-/-)}$ mice. Implications for dietary recommendations on heme intake by Hfe subjects to modulate iron loading are important clinical considerations.

Key words: Hemochromatosis gene; Heme; Gavage; Iron; Absorption

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Core tip: These results indicate that loss of hemochromatosis gene (Hfe) protein results in increased dietary heme iron absorption that further contributes to the iron loading of the liver and other tissues of mice. Enhanced heme iron intake by homozygous Hfe subjects may contribute to body iron overload and early manifestation of phenotypic traits. This may have implications for dietary recommendations on heme intake by hemochromatosis subjects to avert tissue iron loading.

Laftah AH, Simpson RJ, Latunde-Dada GO. Intestinal heme absorption in hemochromatosis gene knock-out mice. *World J Hematol* 2017; 6(1): 17-23 Available from: URL: http://www.wjgnet.com/2218-6204/full/v6/i1/17.htm DOI: http://dx.doi.org/10.5315/wjh.v6.i1.17

INTRODUCTION

Dietary iron intake from both heme and non-heme sources is a key homeostatic step in iron metabolism, of which deficiency or enhanced absorption is associated with iron disorders in populations all over the world[1]. Heme from animal sources contributes about 10%-25% of total food iron and has a higher bioavailability (about 15%-38%) than non-heme iron[2] in humans. The absorption mode and molecular mechanism of both forms of iron are disparate. While non-heme iron is transported by a divalent metal transporter, a proton coupled symporter, heme is presumed to be transited into the enterocytes by endocytosis (passive pinocytosis or active receptor mediation), or proteins[3,4], that are yet to be fully characterised since HCP1 is a high affinity folate transporter[5]. Internalised heme is trafficked from the cytoplasm into endosomes[6] where it is catabolised by heme oxygenase (HO-1) to yield ferrous iron that converges with the labile non-heme iron pool for transit into circulation by ferroportin, the efflux regulatory protein[7]. On the other hand, basolateral efflux of intact heme has been shown in guinea pigs, and this may be *via* Flvcr1 or Abcg2[8].

Hereditary hemochromatosis (HH) constitutes heterogeneous mutations of genes in the hepcidin regulatory pathway. Homozygous C282Y mutation in Hemochromatosis gene (Hfe) is predominant in about 1:300 of Caucasian populations[9]. Coincidentally these populations are, in general, avid consumers of meat and animal products. HH patients are characterized by increased heme and non-heme iron absorption from the diet[10] coupled with excessive iron accumulation in parenchyma cells of the liver and the heart. This occurs because of low hepcidin expression due to loss of function of Hfe[11,12]. Hfe protein is vital for iron-sensing in the signal transduction cascade regulating hepcidin expression. Low serum hepcidin in Hfe patients permits sustained functional expression of ferroportin. Consequently, there is enhanced efflux of non-heme and heme iron by ferroportin into circulation[13,14]. There is, however, disparity in the phenotypic expression of HH which may be due to influences of other modifier genes, dietary factors or physiological iron requirements of the subjects[15]. Consequently, iron loading in HH subjects varies in severity[16,17]. Mouse strains have been shown to modulate phenotypic variability of Hfe severity[13].

Epidemiological studies generally agree that red meat consumption leads to higher iron stores in humans[18,19]. Moreover, dietary heme iron intake was found to be associated with high serum ferritin levels in HH subjects[18]. Of particular interest, however, is the question as to whether Hfe patients could benefit from dietary modifications of iron intake during treatment by phlebotomy.

Further work is needed to elucidate the effects of the loss of Hfe on the regulation of intestinal heme absorption[20]. Mouse knock-out models, however have contributed immensely to significant advances in understanding iron metabolism and disorders. The study, therefore, set out to investigate the effects of Hfe knock-out genotype on heme absorption in mice.

MATERIALS AND METHODS

Reagents

Chemicals and biochemicals were of Analar grade and were from either BDH-Merck Ltd (Poole, Dorset) or Sigma Chemical Company Ltd (Poole). ^{59}Fe (supplied as ferric chloride) was from PerkinElmer Life and Analytical Sciences (Wellesley, MA, United States, specific activity 185 GBq/g). ^{59}Fe-heme was prepared as described in[21]. To make ^{59}Fe-heme, a male Wistar rat was injected ip with 3.7 MBq ^{59}Fe citrate and housed in a metabolic cage for 1 wk. The animal was bled and the red cells washed three times in 10 volumes of saline and then lysed in 10 volumes of distilled water. Heme was then isolated from the haemoglobin by crystallization using the method of Labbe and Nishada[22].

Animals

Animal care and the regulation of scientific procedures met the criteria laid down by the United Kingdom "Animals (Scientific Procedures) Act 1986". Mice were housed in a light- and a temperature-controlled room with *ad libitum* access to standard pelleted diet and water unless stated. Hfe$^{(-/-)}$ breeders (C57/BL6 background strain; donated

by Srai K, Department of Biochemistry and Molecular Biology, Royal Free and University College Medical School, London, United Kingdom) were mated and subsequently genotyped by polymerase chain reaction (PCR). Wild-type and $Hfe^{(-/-)}$ homozygote breeders were established to produce age-matched male mice for experimental study. Mice at 3-5 wk of age were maintained on either iron-deficient (3 mg iron per kilogram) diet *ad libitum* during the treatment with either arginate (control) or heme:Arginate (200 mg/L heme and 3.3 mmol/L arginate) in drinking water for 24 h.

Iron absorption by tied loops in mice

In vivo Fe absorption was measured in tied-off duodenal segments as described previously[23]. In brief, the experiments were conducted in anaesthetised mice. A duodenal segment was tied at both ends followed by the injection of ^{59}Fe-heme arginate (100 μmol/L) into the tied-off segment. The segment was placed back into the abdominal cavity. After 10 min incubation, the duodenal segment was flushed with an ice-cold saline solution and weighed. Blood, liver and spleen were collected. Radioactivity in tissue samples and blood was measured using a gamma counter (1282 Compugamma; LKB Wallac, Turku, Finland), while carcasses were counted for radioactivity by a high-resolution bulk sample counter (J and P Engineering, Reading, United Kingdom). Radioactivity in the duodenum is referred to as mucosal retention while radioactivity in the carcass and other tissues is regarded as mucosal transfer (MT). TMU is the amount of total radioactive Fe absorbed from the gut lumen, and the percentage of MT (% MT) is the relative amount of Fe transfer into the body in comparison with total Fe uptake.

Heme iron absorption in mice after intragastric administration by gavage method

Food was withheld from the mice for 12 h prior to the oral dose, but they had free access to distilled drinking water during that period. Mice were then given 100 μL of physiological solution freshly prepared to contain heme:arginate labelled with 18 kBq ^{59}Fe ($FeCl_3$, in 0.1 M-hydrochloric acid, 1835 MBq/mg Fe; PerkinElmer) to provide target dosages of 4 mmol/kg body weight. This was gavaged as a single dose through the oesophagus and directly into the stomach of the animal through a 40 mm 13 gauge olive-tipped needle. No food was given to the animals after dosing and until tissue collection. The mice were then killed at approximately the same time (of 30 min) after the oral dose was administered. The abdomen was opened and after blood collection *via* a 1 mL syringe through a puncture into the heart, the whole gut was removed, externally rinsed, and divided into the stomach, duodenum, jejunum, ileum, caecum and colon. The lumen of each section was flushed gently with 3 mL of cold saline (9 g sodium chloride/L). Each section and the collected wash were counted for 1 min in a twin channel γ-counter (LKB, Wallac 1280, Helsinki, Finland). The carcass, minus gut, liver, spleen, kidney and blood, was counted in a high-resolution bulk sample counter for 2 min.

In the present study, it was found that 30 min were sufficient time to allow for passage of approximately 50% of the radiolabelled dose through the duodenum. Mucosal uptake of ^{59}Fe-heme measured only in the duodenum and jejunum were defined as the proportion of the initial dose of the label retained by the carcass plus duodenal and jejunal wall after dosing. Mucosal transfer at a given time was defined as the amount of ^{59}Fe in the carcass expressed as a percentage of the mean mucosal uptake[24].

PCR amplification procedures

Total RNA was extracted from tissue samples using Trizol reagent (Invitrogen, United Kingdom) according to manufacturer's instructions. Quantitative RT-PCR was carried out using an ABI Prism 7000 detection system in a two-step protocol with SYBR Green (ABI, Life Technologies, United Kingdom). The efficacy of the amplification was confirmed by a melting curve analysis and gel electrophoresis to confirm the presence of a single product. Quantitative measurement of each gene was derived from a standard curve constructed from known amounts of PCR product. The results were calculated by the ΔCt method that expresses the difference in threshold for the target gene relative to that of 18S RNA.

Statistical analysis

Data are presented as means with their standard deviations. The comparison of multiple groups for significant effects of two variables was determined by two-way ANOVA with a Bonferroni post hoc test. $P < 0.05$ was considered as significant. All statistical analyses were performed using GraphPad Prism 4 software (GraphPad Software, Inc., La Jolla, CA, United States).

RESULTS

Heme absorption

Heme absorption, determined by the tied loop method was greater in $Hfe^{(-/-)}$ knockout mice than in control wild type mice (WT) (Figure 1). This was due to a significant increase in both the uptake and transfer phases of absorption. Moreover, a similar trend of absorption was observed when heme absorption was determined by oral gavage (Figure 2). Following the oral administration of ^{59}Fe-heme, intestinal uptake and transfer were elevated in $Hfe^{(-/-)}$ compared to WT after 30 min of heme administration.

Gene expression studies

To analyze the expression of genes involved in heme metabolism and transport, WT and $Hfe^{(-/-)}$ mice were administered arginate (control) or heme:arginate (200 mg/L heme and 3.3 mmol/L arginate) in drinking water for 24 h. $Hfe^{(-/-)}$ mice treated with heme and maintained on iron-deficient diets for 24 h showed an induction of *HO-1* expression (Figure 3A). The increase in *HO-1*

Table 1 Primer sequences of genes

Forward	Reverse
Mouse Flvcr1	
5′-CAGTTGATAGTCGGGTAGATCCAA-3'	5'-ACACCGGCTTCTTCAGAGTGA-3′
Mouse Abcg2	
5′-TCGCAGAAGGAGATGTGTTGAG-3	5CCAGAATAGCATTAAGGCCAGG-3′
Mouse HO-1	
5′-CAAGGAGGTACACATCCAAGCC-3'	5'-TACAAGGAAGCCATCACCAGCT-3′
Mouse 18S	
5′-GAATTCCCAGTAAGTGGCGGG-3'	5'-GGGCAGGGACTTAATCAACG-3′

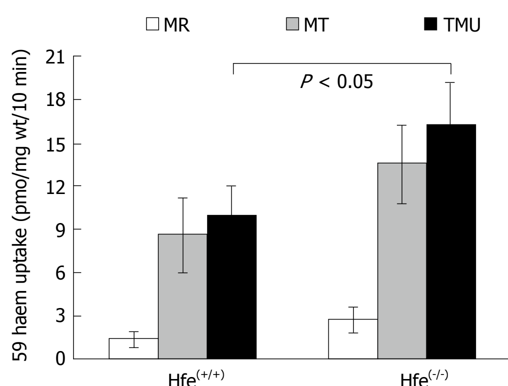

Figure 1 Tied loop mucosal uptake of ^{59}Fe-heme (100 μmol/L) in wild type and hemochromatosis gene$^{(-/-)}$ mice. Iron absorption was determined using tied-off duodenal segments. Data are means ± SD for 5 mice in each group ($P < 0.05$). MR: Mucosal retention; MT: Mucosal transfer; TMU: Total mucosal uptake of ^{59}Fe from *in vivo* tied-off duodenal segments; Hfe: Hemochromatosis gene.

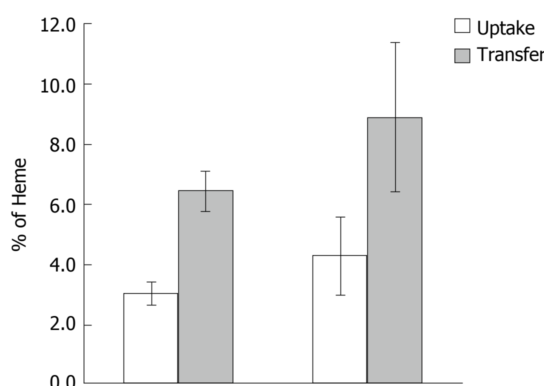

Figure 2 Heme absorption in wild type and hemochromatosis gene$^{(-/-)}$ mice by gavage method. Mice were orally gavaged with ^{59}Fe-heme (100 μmol/L) after an overnight fast. Mice were sacrificed 30 min after the oral dose was administered and tissues were collected as detailed in Methods. Values are mean ± SD, n = 5 per group.

expression in the duodenum of mice on the control iron-deficient diet was not significant (Figure 3A). *Flvcr1* mRNA level was lower in the duodenum of WT than Hfe$^{(-/-)}$ in mice fed the control iron-deficient diet. *Flvcr1* mRNA levels were significantly down regulated after 24 h heme feeding in drinking water (Figure 3B). *Abcg2* mRNA expression levels, however, were not significantly altered by heme feeding in drinking water (Figure 3C).

Serum and tissue iron levels

Serum and tissue iron status was determined in the mice after 24 h of heme feeding. Consistent with the literature, serum iron and transferrin saturation were significantly higher in Hfe$^{(-/-)}$ than WT (Table 1). Contrary to expectation, however, feeding heme to WT or Hfe$^{(-/-)}$ mice for 24 h showed no effect on serum iron and transferrin saturation (Table 2).

Endogenous non-heme iron levels in liver and spleen homogenates from Hfe$^{(-/-)}$ mice were significantly higher than WT (Figure 4; $P < 0.001$). Non-heme iron levels in liver homogenates were not significantly influenced by heme feeding. Although liver showed a trend towards being increased in Hfe$^{(-/-)}$ mice.

DISCUSSION

Heme as an exogenous source of iron is significant in nutrition because it is highly bioavailable for absorption by the gastrointestinal tract. In systemic metabolism, however, heme is derived endogenously from *de novo* biosynthesis for vital metabolic functions. Consequently, modulation of cytosolic, vesicular, membrane or plasma heme transport is regulated by a variety of extracellular and intracellular proteins[25,26]. While the luminal high affinity heme transport protein is not yet defined, heme absorption is enhanced in HH subjects and it is regulated by iron stores albeit by an order of magnitude less than non-heme iron absorption[10,27].

The current study demonstrates that heme feeding stimulates iron absorption in Hfe KO mice and provides evidence of increased iron storage in the spleen and hepatocytes of the mice. ^{59}Fe-Heme arginate absorption from the duodenal loop of the mice was significantly enhanced in Hfe$^{(-/-)}$ mice after 10 min of exposure (Figure 1). This trend was also confirmed in mice that were given ^{59}Fe-heme arginate by gavage and measuring absorption after 30 min (Figure 2). Hfe has been shown to have an impact on cellular iron trafficking and, indirectly, on intestinal iron absorption. The direct effects of Hfe on heme iron absorption are not clear. Alternatively, low levels of iron in the enterocyte of Hfe mice might induce heme absorption *via* increased HO-1 expression. It has been speculated that heme degradation by HO-1 might be the rate-liming step of heme absorption in the gut because the HO-1 activity was found to increase during Fe deficiency[28]. Increased heme iron absorption after

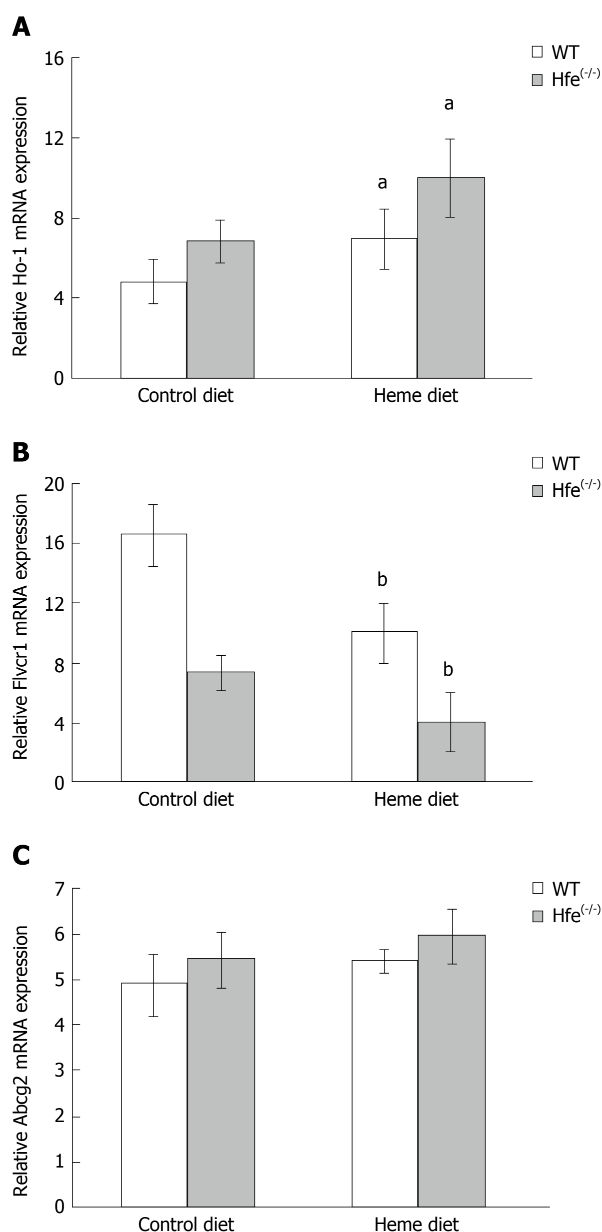

Figure 3 ***HO-1*, *Flvcr* and *Abcg2* mRNA expression in wild type and hemochromatosis gene$^{(-/-)}$ mice fed control diet or heme for 24 h.** Real-time polymerase chain reaction of mRNA of the genes from the duodenum of WT and Hfe$^{(-/-)}$ were determined and normalised β-actin (Actb) mRNA. Statistical analysis was performed by 2-way ANOVA with Bonferroni post-hoc test ($^aP < 0.05$ and $^bP < 0.001$). Hfe: Hemochromatosis gene; WT: Wild type.

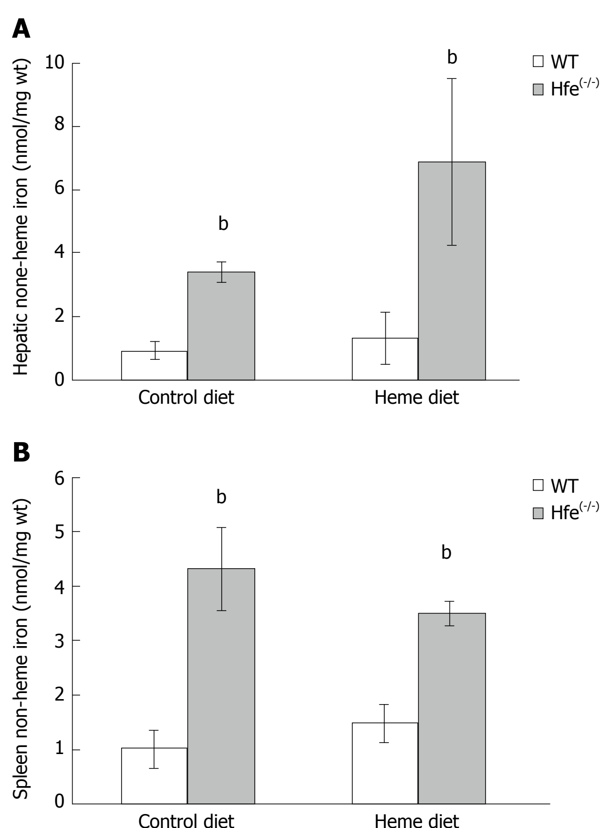

Figure 4 Tissue iron levels of mice. Effect of 24 h heme feeding on liver (A) or spleen (B) non-heme iron levels (nmol/mg) of WT and Hfe$^{(-/-)}$ mice. Results are means ± SD for 6-8 mice in each group ($^bP < 0.005$). Hfe: Hemochromatosis gene; WT: Wild type.

Table 2 Iron parameters of wild type and hemochromatosis gene$^{(-/-)}$ mice after 24 h of feeding heme

		Serum iron (μmol/L)	% Transferrin saturation
WT	Control	25.3 ± 4.4	32.1 ± 6.5
	Heme-24 h	26.8 ± 3.1	35.9 ± 7.9
Hfe$^{(-/-)}$	Control	49.8 ± 5.0	59.8 ± 6.1
	Heme-24 h	50.5 ± 10.6	61.0 ± 10.4

Hfe: Hemochromatosis gene; WT: Wild type.

24 h, shown in Figure 1, might be induced by enhanced expression of *HO-1* (Figure 3A). Augmented catabolism of heme by *HO-1* consequently may increase the inorganic iron pool that can be chaperoned into systemic circulation. It has also been speculated that a fraction of the heme in the enterocyte might be transferred intact into the circulation by the heme efflux proteins Flvcr or Abcg2. The expression of *Flvcr1* was down-regulated in Hfe mice (Figure 3B). Other modifiers such as TfR1 or TfR2 could interact with Hfe directly or, more likely, modify iron loading through independent mechanisms to increase or depress the effects of Hfe. This possibly might be due to hepatocyte regulation of hepcidin expression. A two-fold decrease was observed in hepcidin mRNA levels in the Hfe$^{(-/-)}$ mice used in the current study[29]. Reduced expression of hepcidin in Hfe$^{(-/-)}$ phenotype would lead to the maximal functional capability of FPN, hence the enhanced absorption of iron[30] in Hfe$^{(-/-)}$ mice. Hepcidin levels in the liver correlate negatively with serum ferritin which in humans is a biomarker of iron intake and iron status.

The increase in plasma iron and percentage transferrin saturation after feeding heme for 24 h might have contributed to increased liver and spleen non-heme iron levels in Hfe$^{(-/-)}$. The phenotype of HH patients of European descent attests to the higher iron absorption due to enhanced duodenal expression of transport proteins despite high iron stores[31]. Previous studies have attempted to use low iron intake and inhibitors of iron

absorption as dietary strategies to ameliorate the rate of tissue Fe deposition in Hfe patients[32].

While the feeding of high heme diet did not increase serum and hepatic iron levels of both Hmox1$^{fl/fl}$ and Hmox1$^{Vil\text{-}Cre}$ mice[33], Hfe knock-out mice demonstrated increased *HO-1* expression and enhanced heme absorption in the current study. Mouse strain differences have been shown to determine the severity of tissue iron deposition in Hfe knockout model of HH[13]. There might be species or strain differences in the absorption of heme iron, an earlier study however, showed that mice have the least heme absorption capacity, while canines are the highest (dog > guinea pig > rat > mouse)[34]. Moreover, to sustain heme in solution, heme arginate was used in the current study to measure absorption[35]. This study has identified HO-1 as a key candidate in the regulation of heme iron transport in the gastrointestinal tract of mice. Increased HO-1 expression in Hfe KO mice contributes to enhanced heme iron absorption.

Enhanced heme iron intake by homozygous Hfe subjects may contribute to body iron overload and early manifestation of phenotypic traits. This may have implications for dietary recommendations on heme intake by HH subjects to avert tissue iron loading. Moreover, since high intake of red meat has been associated with an elevated amount of iron in the body and increased risk of metabolic diseases, an emerging consensus, in general, suggests reduced red meat consumption by the populace.

COMMENTS

Background

Hemochromatosis patients are characterized with high level of heme- and inorganic iron absorption from the diet coupled with excessive iron accumulation in parenchyma cells of the liver and the heart due to low hepcidin expression.

Research frontiers

Enhanced heme iron intake by homozygous Hfe subjects may contribute to body iron overload and early manifestation of phenotypic traits. Moreover, since high intake of red meat has been associated with elevated amount of iron in the body and increased risk of metabolic diseases, an emerging consensus, in general, suggests reduced red meat consumption by the populace.

Innovations and breakthroughs

These results indicate that loss of Hfe protein results in increased dietary heme iron absorption that further contributes to the iron loading of the liver and other tissues of mice. This may have implications for dietary recommendations on heme intake by HH subjects to avert tissue iron loading.

Applications

Implications for dietary recommendations on heme intake by Hfe subjects to modulate iron loading are important clinical considerations.

Terminology

HH: Hemochromatosis; Hfe: Hemochromatosis gene; Flvcr1: Feline Leukemia Virus Subgroup C Cellular Receptor 1; HO-1: Hemoxygenase-1; Abcg2: ATP-Binding Cassette, Subfamily G, Member 2; MR: Mucosal retention; MT:Mucosal transfer,TMU:Total mucosal uptake.

Peer-review

It is a very well written manuscript investigating the influence of Hfe mutation on Fe labeled duodenal heme absorption in mice and showing that heme absorption was enhanced from both duodenal tied-loop segments and by oral gavage methods.

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CASE REPORT

Role of plasmapheresis in early allograft dysfunction following deceased donor liver transplantation

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Informed consent statement: All involved persons (subjects or legally authorized representative) gave their informed consent (written or verbal, as appropriate) prior to study inclusion.

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Abstract

The role of plasmapheresis in liver failure and hepatic encephalopathy is undefined and its use as a strategy to salvage patients with severe allograft dysfunction after liver transplantation remains investigational. We present a case of early allograft dysfunction following deceased donor liver transplantation (DDLT) where plasmapheresis was effective as a bridge to recovery and possibly avoiding a retransplantation. A 16 years old boy, known to have decompensated Wilson's disease underwent DDLT at our Public Sector Hospital. He received a healthy liver from a brain-dead donor, whose liver was considered too large for the boy. The graft was reduced *in situ* to a left lobe graft. Surgery was uneventful and the recipient was well for the initial 96 h. On Doppler and further computed tomography scan, a partial portal vein thrombus was noted. He was reexplored and a Fogarty endothombecteomy was performed. Following the second surgery, he developed severe allograft dysfunction with a peak bilirubin of 40 mg/dL. He underwent imaging to rule out technical causes for the dysfunction, followed by a liver biopsy, which revealed acute cellular rejection. Multiple cycles of plasmapheresis were initiated. Over the next two weeks, the graft demonstrated a gradual recovery. He was discharged on the 30th postoperative day, with a serum bilirubin of 5.5 mg/dL. He remains well on follow-up, with the liver function tests improving further. Our report demonstrates the beneficial effect of plasmapheresis, which appears to be an effective treatment option for

early allograft dysfunction following liver transplantation and may obviate the need for retransplantation.

Key words: Liver transplantation; Allograft dysfunction; Plasmapheresis

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Core tip: We demonstrate the beneficial effects of plasmapheresis, which appears to be an effective treatment option for early allograft dysfunction following liver transplantation and may obviate the need for re-transplantation.

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INTRODUCTION

With expanding indications and increasing demand for liver transplantation (LT) donor organ shortage is a major limitation. Early allograft dysfunction (EAD) is not an uncommon entity, especially in transplantation with organs from marginal donors[1]. The incidence of EAD varies between 1.4%-23%, with a median range of 5%-6%[1-3]. This wide range of incidences is attributable to the myriad of definitions which exist for EAD although most definitions are a combination of elevated bilirubin, international normalized ratio (INR), transaminases, and hepatic encephalopathy.

EAD leads to increased morbidity and may result in mortality and liver support therapies need to be instituted[2-5]. In severe forms, retransplantation may be the only treatment modality. If the duration of early graft dysfunction passes uneventfully, the patient often recovers spontaneously[1-6]. Measures like liver support devices lessen the hepatic metabolic burden and may help in the recovery of graft function[2-4]. Plasmapheresis has been used in acute liver failure, but its role in supporting dysfunctional liver allografts remains unclear[7,8].

In this brief report, we present a case of allograft dysfunction following deceased donor liver transplantation (DDLT) where the graft was salvaged using multiple cycles of plasmapheresis.

CASE REPORT

A 16-year-old boy weighing 33 kg, with decompensated Wilson's disease underwent DDLT at our Public Sector Hospital. He received a healthy liver from a 34-year-old brain-dead donor. The donor had an initial Sodium value of 194 meq/L, which was controlled and brought down to 164 meq/L at the time of organ retrieval. The donor had one episode of significant hypotension. As the donor liver was considered too large for the boy, it was reduced in situ into a left lobe graft.

The recipient operation was uneventful with a total blood loss of 1000 mL. The end lactate was 4.2 mmol/L from a peak of 10.2 mmol/L in the anhepatic phase. The total cold ischemia time was 210 min. The graft had an accessory artery from the left gastric artery, taken as a cuff from the celiac axis and anastomosed to the common hepatic artery of the recipient. The surgery was uneventful and the recipient was well initially; being extubated on the 1st postoperative day (POD). Immunosuppression was initiated with steroids (Methyl-Prednisolone 0.25 mg/kg per day) and calcineurin inhibitors (Tacrolimus 0.03 mg/kg per day) from POD 1. On the 5th POD, his drain output increased from 600 mL in 24 h to 1700 mL and his serum bilirubin which had dropped to 3.8 mg/dL, went upto 9.3 mg/dL. Doppler showed poor flow in the portal vein. On further imaging, he was noted to have a partial portal vein thrombus. He underwent emergency re-exploration when a Fogarty endothrombectomy was done and the graft was revascularised with an iliac vein interposition graft for the portal venous anastomosis. During the second surgery, the graft was noted to be very stiff. Following this, he developed severe allograft dysfunction (rising serum bilirubin > 10 mg/dL over 3 consecutive days in the absence of biliary complications). Over the next 5 d his bilirubin increased up to 23.5 mg/dL, while his transaminases remained normal. He underwent repeat imaging which ruled out technical causes for the dysfunction including a patent portal vein. Liver biopsy was performed which was suggestive of moderate acute cellular rejection, there was no evidence of antibody mediated rejection. He received pulsed steroid therapy (Methyl-Prednisolone 20 mg/kg per day on consecutive three days). Despite the steroid pulse, the graft dysfunction did not abate and the hyperbilirubinemia persisted on an upward trend, peaking at 40.8 mg/dL on the 15th POD. In an effort to salvage the graft, plasmapheresis was initiated on the 15th POD.

Plasmapheresis was done on 5 consecutive days using continuous flow centrifugal technology based Spectra Optia Apheresis system (Terumo BCT, Denver, CO, United States). Acid citrate dextrose-A anticoagulation and dual vascular access were used. Patient's total blood volume (BV) was calculated as per Nadler's formula and Plasma volume (PV) was calculated according to the formula PV = BV × (1 - Hematocrit)[8]. 1.0 PV was processed in each session with 100% replacement using 5% albumin solution and blood group specific fresh frozen plasma. The inlet: Anticoagulant ratio was kept 1:12 to 1:15 and blood flow rate kept between 45-50 mL/min. Baseline calcium was monitored before each procedure and 20 mL 10% calcium gluconate was given prophylactically during the procedure to prevent citrate toxicity. Continuous monitoring of pulse and blood pressure was carried out during the procedure to prevent any adverse

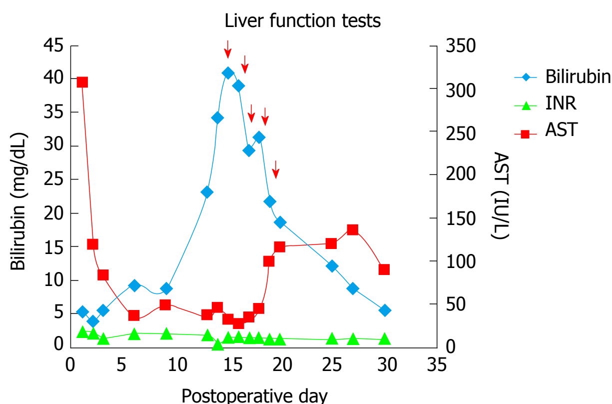

Figure 1 Liver function tests and its response to plasmapheresis. Red arrow: Plasmapheresis; Bilirubin in mg/dL; AST: Aspartate transaminase in IU/L; INR: International normalized ratio.

events related to the procedure. No serious adverse effects were observed during the procedure. Complete blood count, INR, liver function tests, renal function tests, arterial ammonia, arterial blood gas analysis, were performed every 12 hourly irrespective of the timings of the plasmapheresis.

His bilirubin showed a steady fall and by the 5th cycle of plasmapheresis it had dropped to 15 mg/dL (Figure 1). In the interim, he had an episode of fever with chills, and grew *K. pneumonia* in his blood culture. This was successfully treated with appropriate antibiotics (Piperacillin-Tazobactum). No obvious source for the infection could be discerned. He was discharged on the 30th POD being asymptomatic, tolerating oral diet well, with stable vital signs and with a serum bilirubin of 5.5 mg/dL. He remains well on follow-up, with the latest liver function tests showing a total bilirubin of 1 mg/dL, 4 mo after transplantation.

DISCUSSION

Although the pathophysiological basis for early allograft dysfunction has not been wholly elucidated; it appears to be a critical interplay between donor factors, recipient characteristics, and intra-operative events[1,2,4,5].

Despite a few studies including one by Park *et al*[4] having shown plasmapheresis to be beneficial in severe graft dysfunction; the role of plasmapheresis remains undefined in graft dysfunction[1-4]. The mechanisms by which plasmapheresis is beneficial hasn't been completely elucidated, but it does remove the plasma containing free and protein-bound toxic substrates and infuse fresh plasma, as well as clotting factors and albumin, thus functioning as a liver support; creating a milieu conducive to liver regeneration[1-6]. Plasmapheresis is an important adjunct in the treatment of hepatic encephalopathy as it improves blood-clotting, hyperbilirubinemia, and hyperammonemia; acting as a bridge to LT[7,8].

In a series from Japan, all 46 patients with liver failure following LT improved with plasmapheresis[5]. In another recent study by Choe *et al*[3] consisting of 143 patients with EAD of whom 107 underwent Plasmapheresis. There was a significant improvement in the 1-mo and 1-year survival of this subgroup of patients as compared to those who did not undergo plasmapheresis. A report from Johns Hopkin also suggested that plasmapheresis may aid in the recovery of primary allograft nonfunction following liver transplantation[9,10].

As demonstrated in our patient, a single plasmapheresis session cannot be expected to provide a definite beneficial effect in patients with a failing liver graft[2,3]. Repeated sessions appear necessary to achieve cumulative effects. The timing and interval of plasmapheresis must be adjusted on a case-by-case basis, by daily determination of patient's general condition and liver graft function[2-4]. In a dysfunctional liver, the liver enzymes often fluctuate, depending on the condition of the liver graft, and hence cannot be used to assess the effectiveness of plasmapheresis. Prothrombin time is readily affected by the plasma infusion and is also not a predictable marker of the effectiveness of plasmapheresis[3,11]. Serum bilirubin appears to be the most reliable parameter to base decision regarding the initiation, continuation and termination of plasmapheresis[2,4,11].

In countries, where retransplantation may not be a feasible option due to the lack of availability of donor grafts and/or the huge financial burden involved, plasmapheresis appears to be a readily available artificial liver support system with the added advantage of being economical, simple and easy to use.

Apart from benefiting the patient, effective management of early allograft dysfunction helps a unit improve its overall efficiency and sets a benchmark for excellence in care by showing an enhancement in healthcare delivery in general.

In conclusion, our report demonstrates the beneficial effect of plasmapheresis, which appears to be an effective treatment option for early allograft dysfunction following DDLT and may obviate the need for retransplantation.

COMMENTS

Clinical diagnosis

Early liver allograft dysfunction.

Differential diagnosis

Arterial complications, venous complications, biliary complications, rejection, infection.

Laboratory diagnosis

Early liver allograft dysfunction.

Imaging diagnosis

Early liver allograft dysfunction.

Pathological diagnosis

Early liver allograft dysfunction.

Treatment

Plasmapheresis.

Term explanation

Plasmapheresis - total plasma exchange.

Experiences and lessons

Useful not to disregard a simple but very effective procedure such as plasmapheresis in treating early allograft dysfunction.

Peer-review

The paper is good, although it doesn't seem to get different conclusion from the larger case series already published.

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