2.5.2.2 Apheresis method Granulocyte collection may usually be performed on various blood cell separators using the white blood cell cytapheresis set and an exclusive program of the separator's software. It was reported that the use of higher interface offset settings (35 vs. 15) resulted in a significant increase in the granulocyte collection efficiency [19]. Because interface offset settings are dependent on the apheresis systems used, the relevant setting should be evaluated and used for achieving a maximal granulocyte yield in the hospital. The required apheresis procedure for granulocyte collection would present a potential clinical risk for cardiac or cerebrovascular events in donors with preexisting inflammatory or vascular disease, and, as such, should be avoided in these subjects.

2.5.3 Preparation of granulocyte concentrates

2.5.3.1 Gamma irradiation Granulocyte concentrates contain significant amount of donor lymphocytes and are frequently transfused to immunocompromised patients with neutropenia [20]. Currently, the gamma irradiation of blood components is the only proven effective method for TA-GVHD prevention [20]. The AABB Standards recommend a minimum 25 Gy dose of gamma irradiation to the central portion of the container, with no less than 15 Gy delivered to any part of the bag [21]. 'HLA one-way match' results in the inability to reject donor lymphocytes even if the recipient is immunocompetent, and it occurs at a rather high frequency, one in several hundred blood transfusions from unrelated donors in Japan [22]. The JRCBC disseminated transfusion information regarding TA-GVHD to most Japanese hospitals in December 1999, in which the administration of irradiated blood components except for fresh-frozen plasma is recommended for preventing TA-GVHD. Most Japanese hospitals are generally supplied with 15-Gy (or more)-irradiated blood components from branches of the JRCBC. If hospitals have an exclusive gamma-irradiation apparatus for blood, non-irradiated components are supplied and irradiated at a dose between 15 and 50 Gy in transfusion services [23]. Thus, granulocyte concentrates should be irradiated before administration to the patient at a dose between 15 and 50 Gy. Recent studies have demonstrated that the irradiation of neutrophils did not affect their in vitro functions, including respiratory burst activity and phagocytosis [24].

2.5.3.2 Storage There is general agreement that granulocyte concentrates should be administered as soon as possible after collection [21]. The British Committee for Standards in Haematology (BCSH) recommended that granulocytes should be stored in the same donor's citrate-anti-coagulated plasma at room temperature, kept unagitated, and administered within 12 h of preparation [25]. In the case of a limited number of available granulocyte donors, there may be a need for storage of an aliquot of granulocyte concentrates. G-CSF has been shown to inhibit granulocyte apoptosis [26], and may be useful in lengthening the acceptable storage time for granulocyte concentrates and, thereby, improving the logistics of GTX programs [9]. Drewniak and colleagues [27] investigated granulocytes from leukapheresis products mobilized by G-CSF with DEX, where in vitro granulocyte functions were intact at least 24 h. Mochizuki and colleagues [18] also reported the extended storage of granulocyte concentrates mobilized by G-CSF with or without DEX, where in vitro granulocyte functions were maintained for as long as 72 h after collection by the 'bag separation method'. The current guidelines recommend that granulocyte concentrates should be transfused within 48 h after collection.

2.6 Administration of granulocyte concentrates

2.6.1 Infusion of granulocyte concentrates

Granulocyte concentrates should be slowly administered over 1–4 h through a standard transfusion set with a screen filter (170–200 μm) within 6 h after collection. In the case of 200 ml of granulocyte concentrates, it should be administered over 1– 2 h in adults and 2–4 h in pediatric patients. In general, granulocyte concentrates are administered every other day until complete recovery from infection is documented. The BCSH guidelines recommend that all granulocytes should be irradiated for patients of any age and transfused as soon as possible after irradiation [25]. Leukocyte reduction filters must not be used, because it makes no sense to use these filters in GTX. Patients should be monitored by pulse oximetry. The blood pressure should also be measured every 15 min during the infusion of granulocyte concentrates.

2.6.2 Premedication

The administration of antipyretics or corticosteroids (e.g., 100 mg of hydrocortisone) is appropriate for patients who experience symptoms such as chills and fever. Routine prophylaxis with these agents is not necessary [9].

2.7 Evaluation of effectiveness of GTX therapy

2.7.1 Success of GTX therapy

The success of GTX therapy is defined as complete recovery from infection, being documented by: (a) disappearance of clinical symptoms (e.g., fever), (b) negativity of laboratory findings (e.g., C-reactive protein), (c) disappearance or

marked reduction of radiological findings, or (d) negativity of microbiological cultures.

2.7.2 Discontinuation of GTX therapy

In general, GTX therapy is continued daily to maintain an ANC of more than 500/μl until neutrophil recovery, clinical improvement, or stability. However, prolonged GTX therapy may be difficult in cases of a limited number of available granulocyte donors. The current guidelines recommend criteria for the discontinuation of GTX therapy as follows: (a) neutrophil recovery or bone marrow engraftment with an ANC of more than 500/μl in patients who received HSCT, (b) recovery from infection without the need of GTX support, (c) refractoriness to GTX therapy even if continued for 7 consecutive days, or (d) occurrence of an adverse event due to GTX therapy.

2.8 Complications of GTX therapy

2.8.1 Donor-associated side effects

2.8.1.1 G-CSF Short-term side effects: The most commonly reported side effects of G-CSF administration include bone pain, headache, fatigue, nausea, fever (with or without chills and sweats), insomnia, anorexia, and myalgias [28]. All side effects appear to be generally mild and usually resolve after the discontinuation of G-CSF. However, analgesics may be needed for bone pain, which was the most frequent symptom [29, 30]. Suggested contraindications to G-CSF administration in donors include the presence of active inflammatory conditions and hypercoagulable states, with or without previous venous thrombosis and known or suspected atherosclerotic vascular disease [28].

Long-term side effects: The question regarding the long-term safety of G-CSF administration to normal donors, particularly in terms of the leukemogenic potential, has been raised. Theoretically, a prior history of malignancy or a strong family predisposition to acute myeloid leukemia (AML) or myelodysplasia may place individuals at a higher risk of developing hematologic malignancies [14]. There are limited data generated by long-term follow-up studies on normal donors who received G-CSF administration for granulocyte collection. Quillen and colleagues [31] recently reported 2 cases of lymphoid malignancy (one case each of non-Hodgkin's lymphoma and chronic lymphocytic leukemia) in 83 unrelated granulocyte donors who received repeated administrations of both G-CSF and DEX and were followed for a median of 10 years. Although it has been shown that pharmacologic doses of G-CSF affect cytokine production by lymphocytes in vitro and in vivo [32], there is no evidence to date supporting an association between G-CSF and lymphoid malignancy [31]. Bux and colleagues [16] reported on a 2-year follow-up of 183 granulocyte donors, where no severe G-CSF-related adverse events were noted. The Research on Adverse Drug Events and Reports (RADAR) project reviewed clinical literature on adverse events that occur when G-CSF is administered to healthy individuals for PBSC collection [29]. Three PBSC donors were described who developed AML following stem cell mobilization, but the evidence supporting causality is unclear.

2.8.1.2 Corticosteroids It remains controversial whether the administration of corticosteroids along with G-CSF stimulation to granulocyte donors increases the risk of posterior subcapsular cataract [33, 34]. However, the administration of corticosteroids to granulocyte donors, especially in frequent donations, should be used with caution.

2.8.1.3 RBC-sedimenting agent RBC-sedimenting agents, such as high-MW HES, act as a plasma expander and can cause transient hypertension with flushing and headache. Severe itching following the infusion of HES may be observed in a small number of granulocyte donors [16].

2.8.1.4 Apheresis donation During apheresis, anticoagulation is necessary to prevent coagulation and the clumping of collected components. CPD is returned to the donor, and its toxicity occasionally causes symptoms associated with decreased ionized calcium levels (e.g., peri-oral paresthesia). As an antidote to citrate toxicity, calcium prophylaxis may be required during large-volume leukapheresis.

2.8.2 Recipient-associated side effects

2.8.2.1 Transfusion reactions Mild to moderate fever and chills are relatively common, whereby the slowing of administration may be required. These reactions are preventable on subsequent transfusions by treatment with antipyretics or corticosteroids [12]. However, routine prophylaxis with these agents is controversial. More severe reactions may occur in approximately 1–5% of cases of GTX therapy, including hypotension, pulmonary infiltrates, and respiratory distress [35]. In patients who receive repeated GTX therapy, alloimmunization and platelet refractoriness may develop [36]. The rate of leukocyte alloimmunization has been reported to be 24% [16].

2.8.2.2 Concurrent use of Amphotericin B Although an association between pulmonary infiltration and Amphotericin B administration has not been confirmed [37], it is still common practice to separate the administration times if Amphotericin B and granulocyte concentrates are being given concurrently [35]. The current guidelines recommend that granulocyte concentrates should be administered

at least 4 h after stopping Amphotericin B administration in patients who receive both Amphotericin B and granulocyte concentrates.

3 Conclusion

The current guidelines may be appropriate for the clinical situation in Japan, in which granulocyte donors cannot be selected from the community pool of apheresis donors of the JRCBC, and high-MW HES products are not approved. Care should be taken to perform GTX therapy considering the safety management of both granulocyte donors and patients. Future randomized controlled trials are needed to clarify the efficacy of GTX therapy and identify which subgroup of patients benefits the most.

Conflict of interest statement The authors declare no conflicts of interest.

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