3 Granulocyte Concentrates

3.1 Preparation
   3.1.1 Quality Criteria

3.2 Active Constituents

3.3 Physiological Function

3.4 Storage and Shelf Life

3.5 Range of Application, Dosage, Mode of Administration
   3.5.1 Indications
   3.5.2 Special Indications
   3.5.3 Dosage
   3.5.4 Mode of Administration
   3.5.5 Refractoriness

3.6 Adverse Reactions

3.7 Documentation

3.8 References
3.1 Preparation

Granulocyte concentrates (GCs) are prepared from the blood of healthy donors by automatic apheresis and are therefore also known as granulocyte apheresis concentrates. To ensure sufficient granulocyte content, donors are pretreated with corticosteroids and/or recombinant growth factors for granulocytes (granulocyte colony-stimulating factor; G-CSF). This pretreatment with G-CSF significantly increases granulocyte yield [5, 7] and prolongs granulocyte survival time [15]. To achieve better separation of granulocytes from erythrocytes, during apheresis the collected blood is supplemented by a sedimenting agent, usually 6% high-molecular-weight hydroxyethyl starch (HES) [6]. Because of the risk of severe itching following infusion of HES, granulocyte donations are limited to four per donor and year [6, 10]. The specifications of article 9 of the German Transfusion Act (Transfusionsgesetz; TFG) must be observed regarding pretreatment of donors with G-CSF. Pretreatment of donors with G-CSF should only be performed in the context of notified mobilization programs so that, in case late adverse reactions occur, all pretreated donors can be reached quickly for clarifying follow-up examination.

Regarding donor suitability as well as requirements of product quality, the national and European laws and directives mentioned in chapter 1 are referred to.

3.1.1 Quality Criteria

GCs must contain a sufficient number of functional neutrophil granulocytes, depending on the weight or rather the body surface of the recipient (see section 3.3). Every GC must be subjected to visual quality control immediately before transfusion. Special attention must be paid to bag integrity, coagulation, aggregate formation, discoloration and hemolysis. GCs in any way suspicious may not be transfused. Furthermore, complete labeling, correct assignment to the patient and expiration date of the preparation must be checked.

3.2 Active Constituents

The effective components of GCs are morphologically and functionally intact neutrophils. Mononuclear leukocytes present in GCs possibly contribute to the anti-infective effect attributed to GCs [14]. Platelets that are often contained in abundance in GCs can alleviate an accompanying thrombocytopenia in the patient. Residual amounts of plasma, anticoagulants, sedimentation accelerators and erythrocytes have no clinical impact.

3.3 Physiological Function

Neutrophils are essential for innate immunity. Their main functions are phagocytosis and elimination of microorganisms. Premedicating donors with growth factors for granulocytes significantly enhances antimicrobial activity of granulocytes [21]. Immediately after transfusion a portion of granulocytes first settles temporarily in the pulmonary circulation, causing transfused granulocytes to appear in their entirety in the peripheral blood with a delay of 1–2 h, where recovery amounts to around 30–50% [16]. Further temporary pooling takes place in spleen and liver. The increase of granulocyte count in peripheral blood following GC transfusion varies considerably with dosage and recipient and may entirely be lacking in conditions with high granulocyte consumption. Normal physiological half-life of granulocytes is 5–9 h which is greatly reduced during febrile processes. Granulocytes collected from donors premedicated with G-CSF have a prolonged half-life [8]. Transfused granulocytes migrate from blood vessels into the infected areas and, following a chemotactical gradient, arrive at the focus of infection where they phagocytize and kill the invading microorganisms [1].

3.4 Storage and Shelf Life

Because of the neutrophils’ autolytic tendency ex vivo, GCs should be transfused as soon as possible after preparation. However, without agitation GCs can be stored at room temperature for a maximum of 24 h without significant loss of their functionality [13, 23].

3.5 Range of Application, Dosage, Mode of Administration

3.5.1 Indications

Numerous case reports and phase-II studies reported a beneficial effect of GC transfusion [19, 20].

A meta-analysis of the therapeutic efficacy of GC transfusion in patients with bacterial sepsis, evaluating 7 controlled clinical studies of adults and 4 studies of neonates, also found significant (p < 0.05) benefit when adequate doses of granulocytes were transfused (see below) [26]. Another meta-analysis of 8 randomized controlled trials, involving 310 patients with neutropenia who were treated with GC transfusions, confirmed the benefit (RR = 0.64) regarding mortality when taking 6 of 8 trials into account. However, there was evidence of significant statistical heterogeneity in the trials [24]. If only the results of the 4 studies transfusing granulocyte doses greater than $1 \times 10^{10}$ were taken into account, the data indicated a significant benefit (RR = 0.37). Regarding rates of reversal of
Infection, the analysis of data from 4 studies found the combined RR of 0.94, again with evidence of heterogeneity.

In spite of the benefit reported for GC transfusion, due to the heterogeneity and small size of the clinical studies available, no well-founded, generally valid conclusions can be drawn from the analysis of the studies regarding the significance of GC transfusion for patients with neutropenia and infection.

The same applies to GC transfusion in neonates with sepsis and neutropenia. Meta-analysis of 3 comparable studies involving a total of 44 patients showed no significant reduction of mortality in favor of GC transfusion compared with placebo or no GC transfusion [17].

Based on their personal experience, clinicians increasingly hold the opinion that, along with the administration of an adequate amount of neutrophils, the particular time of starting the transfusion plays an important role in the outcome of a GC transfusion, i.e. the timely start of GC transfusion as opposed to using it as last resort in the course of a life-threatening infection [12].

A randomized study of prophylactic granulocyte transfusion reported a significant reduction in the number of febrile days and i.v. antibiotics required [2]. A meta-analysis of randomized controlled trials on the efficacy of prophylactic GC transfusion published between 1970 and 1995 showed that a significant reduction in mortality can be achieved by transfusing adequate amounts of granulocytes that were tested normal in serological compatibility tests [27].

Patients with progradient infections and severe neutropenia of less than 500 neutrophils/μl blood, despite optimal antibiotic and antimycotic therapy for more than 48 h, can be candidates for granulocyte transfusion, provided that these infections can become life-threatening due to the nature of the etiological agent and the expected duration of the neutropenic state. The same applies to patients with neutropenia of <500 neutrophils/μl and a high risk of acquiring a life-threatening bacterial or fungal infection. In view of the strain involved for the donor (premedication, infusion of HES, time-consuming apheresis) and the lack of more recent randomized application studies, GCs should preferably be administered predominantly in the context of studies.

### 3.5.2 Special Indications

Patients suffering from one of the rare hereditary disturbances of granulocyte function, such as chronic granulomatous disease, might profit from a granulocyte transfusion in progradient life-threatening infections even when the absolute granulocyte count in peripheral blood is within normal limits [29].

### 3.5.3 Dosage

Investigations involving animal experiments suggest that per GC a minimum of $1.5 \times 10^8$ granulocytes/kg body weight shall be transfused for anti-infective therapy [4]. Meta-analysis of controlled clinical studies showed a significant beneficial outcome if adults received $>1 \times 10^8$ granulocytes/kg body weight and neonates with bacterial sepsis $>0.5 \times 10^7$ granulocytes/kg body weight [26].

The transfusion frequency varies depending on the individual case and on the clinical state of the patient as well as on the efficacy and compatibility of the transfused granulocytes. The transfusion frequency reported ranges between twice daily in acute severe infections and twice per week as prophylactic transfusion after stem cell transplantation [2, 19].

The effectiveness of a granulocyte transfusion is assessed according to clinical criteria and by determining the increase in the number of granulocytes circulating in peripheral blood 2–4 h after completing transfusion (increment).

The increase in granulocyte count in peripheral blood following GC transfusion varies considerably, depending on the dose and on the recipient, and may completely fail to appear if granulocyte-consuming processes occur. On physiological grounds, the half-life time in the blood is around 7 h but is substantially shorter in case inflammatory processes occur.

When success is considered inadequate (increment $<500 \times 10^9/μl$), especially in prophylactic transfusions, alloimmunization of the recipient against HLA- and granulocyte-specific antigens should be excluded.

### 3.5.4 Mode of Administration

Because of the large numbers of contaminating donor erythrocytes, granulocyte preparations should be transfused ABO- and RhD-compatible. A cross-match must be performed. To prevent pulmonary transfusion reactions and reduced transfusion efficiency, a leukocyte compatibility test is required [3, 22]. Older publications have claimed that there was a correlation between the simultaneous administration of amphotericin B and granulocyte transfusions and the occurrence of pulmonary transfusion reactions. To avoid this, it has become
established practice to observe an interval of 4–6 h between the administration of amphotericin B and GC transfusion, even if this correlation has later been challenged [9].

Because a case of fatal graft-versus-host disease (GVHD) was reported in the context of a transfusion of granulocytes [11], GCs must be irradiated prior to transfusion with 30 Gy.

To prevent immunization, RhD-negative women of reproductive age should be given anti-D immunoglobulin (10 µg anti-D/ml erythrocyte sediment) prophylactically if the transfusion of RhD-positive GCs is unavoidable.

CMV transmissions have also been reported in the context of GC transfusions [28]. Therefore, when used therapeutically, it is recommended to give CMV-seronegative GCs from donors who were tested negative for CMV to seronegative recipients [18].

GC transfusion is performed using a transfusion set with standard filter (standardized according to the Act on Medical Devices, pore size 170–230 µm).

Since immediately after transfusion a portion of granulocytes first settles in the pulmonary circulation, causing the transfused granulocytes to appear in the peripheral blood with a delay of 1–2 h (recovery around 30–50%) [16], a slow transfusion rate is recommended (e.g. 1 × 10¹⁰ cells/h) [10], although it was reported that GC transfusions over 35–60 min were well tolerated [19].

3.5.5 Refractoriness

Refractoriness is defined as the repeated absence of an adequate post-transfusion increase in granulocytes. The origins of refractoriness can be immunological or non-immunological. Non-immunological refractoriness can be caused among other things by high fever, sepsis, splenomegaly, or antibiotic therapy. Immunological refractoriness must be anticipated especially in polytransfused patients and multiparous women. Its origin may be alloimmunization against HLA class I antigens or other granulocyte antigens (human neutrophil alloantigens, HNA). The frequency of alloimmunization against leukocyte antigens after repeated GC transfusion varies between 20–30% in the case of iatrogenic neutropenia and up to 80% in patients with aplastic anemia and chronic granulomatous disease [6, 20, 25]. In such cases of immunological refractoriness, HLA and/or granulocyte-antigen-compatible granulocytes are to be transfused.

3.6 Adverse Reactions

GCs from donors pretreated with G-CSF is tolerated well [6]. Fever, chills, and skin irritations are the most frequently observed reactions. The triggering of a severe, especially pulmonary transfusion reaction that has often been reported in the past in the context of a granulocyte transfusion has become an extremely rare event today when the leukocyte compatibility test has no pathological results. Additional adverse reactions that may principally occur in the context of a blood transfusion are listed in chapter 11.

3.7 Documentation

See chapter 1.

3.8 References