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Fetal cells from cord blood as stem cell source: current status and possible implications in gynaecologic oncology

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Summary

Umbilical cord blood is increasingly used as a source of hematopoietic stem cells for transplantation. Due to recent success, cord blood banks are being set up. We reviewed the currently available literature concerning cord blood collection, storage and transplantation, the impact of prenatal and perinatal factors and collection techniques on the quantity and quality of cord blood, and the ethical, legal and social questions related to cord blood transplantation.

Possible implications in gynecologic oncology are reviewed and discussed. The emerging therapeutic use of cord blood for transplantation and transfusion implies new challenges for the speciality of gynecology and obstetrics.

Key words: Cord blood; Hematopoietic stem cells; Transplantation.

Fetal hematopoietic stem cells from umbilical cord blood

Umbilical cord blood (placental blood) is rich in hematopoietic stem cells [1]. This phenomenon can be explained by the ontogenetic development of the hematopoietic system in the human fetus which is characterized by sequential changes of the hematopoietic site from the yolk sac and the aorta-gonadal mesonephros region in the embryo to the fetal liver and then to the bone marrow as the definitive hematopoietic organ [2, 3]. At the end of gestation, hematopoietic stem cells and progenitor cells are therefore still abundant in fetal blood (cord blood). These cells can be obtained after delivery of an infant by harvesting blood from the remaining umbilical cord and the placenta, which are usually discarded [4].

The use of umbilical cord blood as a source of hematopoietic stem cells for transplantation has a number of advantages compared to bone marrow [5]. These include a riskless, non-invasive collection procedure and a virtually unlimited number of potential donors including ethnic minorities (which are currently underrepresented in bone marrow donor registries). Furthermore, frozen cord blood is readily available for transplantation if a recipient matches the HLA-type, while the time between matching and transplantation of bone marrow donors usually takes several weeks. The number of cytomegalovirus infections in a newborn donor is lower than in bone marrow donor populations. Most importantly, as shown recently, the incidence of severe graft-versus-host disease after transplantation is smaller [6]. Even if the reason for this is not yet fully understood, plausible explanations are available [7].

Besides the possibility of genetic diseases in the donor not yet apparent at birth, the main disadvantage is the limited amount of cord blood available from a single donor. Despite a higher proliferative capacity of cord blood progenitors as compared to bone marrow [8], there is still a lower cell number threshold per kg recipient weight, and many cord blood samples do not contain enough cells for transplantation in adults. Many studies have therefore been done with cord blood to explore its ex vivo expansion potential. Recent advances in cell culturing techniques, including new growth factors like thrombopoietin, have improved results of progenitor/stem cell expansion experiments [9]. However, while a certain expansion of early progenitors and even stem cells seems possible in vitro, this process is still limited and not yet ready for clinical use because it usually leads to a certain loss of multipotency and self-renewal capacity of stem cells [10].

Another potential disadvantage might be a diminished graft-versus-leukemia effect based on the lower immunogenicity of T-cells in cord blood, concomittant to the lower rate of graft-versus-host disease. So far however, clinical data to prove or disprove this theory are still lacking.

Cord blood stem cell transplantation

Autologous and allogeneic transplantation of hematopoietic stem cells has been successfully used for decades in the treatment of malignant disorders including leukemia, lymphoproliferative disorders and solid tumors, and non-malignant disorders such as genetic diseases, aplastic anemias or, most recently, autoimmune disease [11, 12, 13]. In recent years, the treatment of gynecological malignancies including breast and ovarian cancer with high-dose chemotherapy and transplantation of autologous stem cells is under investigation.

While there is a clear increase in the number of stem cell transplantations [11], there is a lack of stem cell donors. Alternative sources of stem cells for transplantation are therefore important. Early studies show that the amount of hematopoietic stem cells in cord blood (placental blood) is sufficient for hematopoietic reconstitution after transplantation into allogeneic recipients and can therefore be used as an alternative source of stem cells, in addition to bone marrow and mobilized peripheral blood stem cells [5]. Since the first transplantation of umbilical cord blood in a child with Fanconi anemia was successfully carried out in 1988 [14], an increasing number of related [15] and non-related allogeneic cord blood transplantations for malignant and non-malignant diseases have been performed [16, 17]. The largest series now show comparable results with bone marrow cell transplantation, except a smaller incidence of severe graft-versus-host disease [6]. Most cord blood transplantations, however, have been performed in children due to the limited amount of cells available from one cord blood sample. Cord blood from HLA-identical siblings is now established as the preferential source of stem cells in related transplantations. Clinical trials will have to prospectively compare the short- and long-term outcome of related and unrelated cord blood stem cell transplantation to bone marrow and peripheral blood stem cell transplantation for malignant as well as non-malignant disease. Large scale clinical trials are currently underway, aiming to answer these questions [18].

Banking of umbilical cord blood

As outlined above, the most important limitation for allogeneic stem cell transplantation is the limited availability of histocompatible donors. Only for about 40% of all patients in need of an allogeneic stem cell graft, a HLA-matched donor can be found. Based on the promising results of initial cord blood transplantations, many centers around the world have therefore begun to build up public banks with cryopreserved cord blood [19, 20]. The largest bank of this kind is located at the New York Blood Center where over 10,000 samples of frozen HLA-typed cord blood are stored; other large banks exist in Düsseldorf, Milan, Paris, Madrid, etc. The cord blood banks are coordinated by an international registry through which searches for certain HLA-types can be carried out.

In addition to these public, worldwide accessible banks of anonymously donated cord blood, some companies have set up private cord blood banks. They offer – by direct marketing – future parents the storage of cord blood from their newborn. This implies the collection and cryopreservation of cord blood from healthy newborns from a healthy family for possible later autologous (or allogeneic) use. From a scientific point of view this procedure is controversial and not recommended at this time due to poor cost-effectiveness and lack of solid scientific data. The main argument against "private banking" is that the probability that the stored cells will ever be used for the donor himself or for a family member is extremely small. Nevertheless, these banks operate with considerable success, mainly in the United States: to date, several thousands of cord blood samples are stored for possible future use by the donor or his family.

Collection of cord blood

Recent data derived from the increasing experience with related and unrelated cord blood hematopoietic stem cell transplantation in children and adolescents show that the clinical outcome of cord blood transplantation depends on the number of nucleated cells transplanted [16, 17, 21]. The yield of stem cells remains therefore an important aim of cord blood collection, besides donor (i.e., maternal and neonatal) safety.

The amount of fetal blood in the placenta and the umbilical cord after clamping and dissection depends on several factors. It has been shown that birth weight of the newborn and placental weight determine blood volume [22]. Timing and location of cord clamping influence the redistribution of the total fetoplacental blood volume between newborn and placenta. "Early" clamping (within 30 seconds) leads to increased placental blood volume, whereas "late" clamping favors a greater transfer of blood from placenta to the fetus [23]. Other factors such as placental vasculature, fetal circulation or duration of labor are likely to be involved as well; the influence, however, is less clear. Immediate cord clamping is therefore a possible method to increase the amount of obtainable cord blood; however, the timing of cord clamping can have an impact on the circulating blood volume of the newborn and is therefore a matter of debate. Early clamping might avoid extensive placental transfusion to the newborn and thus decrease polycythemia, hyperbilirubinemia and hypervolemia [24]. Late clamping might avoid newborn anemia and has been shown to be advantageous in preterm infants [25]; this study however has been criticised as being small and not excluding possible biases, including lack of blinding of caring physicians [26]. The issue of the timing of cord clamping has now become a new actuality through the use of cord blood for allogeneic stem cell transplantation. Concern has been raised about possible risks for the newborn involved in cord blood collection for stem cell transplantation at birth if the cord is clamped early to increase collection volume [27, 28]. It seems clear that there is hardly an ethical justification to modify the timing of cord clamping for cord blood collection and banking purposes, potentially putting the newborn at risk. Thus, modifying the clamping time does not seem an adequate measure to increase the collected blood volume. This has also been clearly expressed in a recently published FIGO committee report [29].

For large scale cord blood banking it is mandatory that a standardized collection method is established which leads to reproducible and comparable results. The strategy of umbilical cord blood collection, however, varies between different cord blood banks [30]. "Open" systems which were initially used for cord blood retrieval have been replaced by closed systems using blood collection bags to minimize the risk of contamination [31]. Some authors recommend cord blood collection after delivery of the placenta, whereas others favor the collection while the placenta is still in utero [32]. Other means to increase cord blood yield include multiple punctures, flushing or placenta manipulation [33, 34]. However, these techniques might increase the risk of bacterial, viral or maternal blood cell contamination and could therefore be harmful for the recipient of the cord blood graft (infection or graft-versus-host disease mediated through maternal alloreactive T-lymphocytes). Randomized controlled trials have now shown that cord blood collection before placental delivery is significantly better than after placental delivery in terms of the obtainable blood volume and mononuclear cell counts [35, 36]. Based on these results and previously published data, umbilical cord blood collection from the placenta in situ seems superior to increase the hematopoietic progenitor/stem cell yield without putting the mother or the newborn at risk. Since recent data indicate that cord blood drainage before placental delivery at vaginal birth is beneficial because it reduces maternal blood loss during the third stage of labor [37], maternal safety does not seem to be a concern if cord blood is collected before placental expulsion. While it remains a matter of debate which collection technique is optimal regarding cord blood yield, it seems important that the method chosen is easily applicable in daily routine care by attending obstetricians and midwifes.

Influence of fetal and perinatal factors and maternal cells in cord blood

In the last few years a growing number of papers have been published on hematological and immunological properties of cord blood and the influence from fetal and obstetrical factors. For example, recent studies suggest that prenatally circulating fetal blood is very rich in progenitor cells capable of multilineage differentiation and long-term survival in vitro [38, 39].

Since contamination of cord blood with maternal cells may contribute in an unacceptable way to GvHD when used for an unrelated recipient, sensitive PCR- and FISH-based methods have been established to search for the contaminating cells [40, 41]. In these studies it has been shown that many cord blood samples contain a small amount of maternal cells, although this amount is unlikely to be associated with GvHD in a cord blood graft recipient. An analogous issue is relevant for preterm cord blood but data on cord blood obtained from preterm deliveries or second trimester are scarce [42], and it is not yet defined which cells in

which quantity are involved in maternal-fetal cell trafficking throughout gestation. Furthermore, transplacental cell-trafficking during pregnancy has been shown to be influenced by diseases of gestation like preclampsia [43].

Ethical issues in cord blood banking and transplantation

Similar like every novel technique, especially in transplantation medicine, cord blood collection, banking and transplantation imposes social, psychological, ethical and legal challenges for researchers as well as health professionals [44-46]. It is mandatory that such issues are being explored and discussed before decisions about large-scale cord blood banking are made [47]. In particular if cord blood is used for allogeneic unrelated banking and transplantation, important questions about ownerhip, donor consent (newborn!) as well as systematic storage of human tissue [46] arise and must be evaluated under socio-medical, legal and ethical aspects [48]. These questions are important not only for the public in general but also for the parents confronted with the possibility of donating the cord blood of their child. Generally, cord blood is regarded as discarded tissue. Nevertheless, the mother might have particular emotional or religious views of the placenta and its contents which are not compliant with cord blood collection and donation. Possible differences between ethnical groups might be of particular importance since one of the main advantages of cord blood as a stem cell source is that it could compensate for the under-representation of ethnical minorities in bone marrow donor registries. In a prospective survey among pregnant women from different ethnical origins, we found a high (>90%) acceptance rate of umbilical cord blood donation for banking and stem cell transplantation purposes irrespective of ethnical or religious background [49].

Cord blood stem cells as targets for postnatal gene therapy

Considerable focus has been placed on stem cells as targets for gene therapy [50]. Gene therapy using autologous stem cells is a strategy to circumvent the limitations of allogeneic stem cell transplantation. Hematopoietic stem cells are attractive targets for somatic cell-based gene therapy because they have the potential to continue producing progeny cells containing a lifelong therapeutic gene. Current clinical protocols concern postnatal gene therapy in pediatric patients by ex vivo retroviral transduction of hematopoietic stem/progenitor cells from cord blood or bone marrow, followed by autologous transplantation of the engineered cells back into the patient. Although the feasibility and safety of gene therapy using cord blood cells in patients with ADA-deficiency has been shown, only very limited clinical efficiency has been achieved in initial trials [51]. Only very recently, successful gene therapy in X-linked SCID (severe combined immunodeficiency syndrome) using retrovirally transduced autologous bone marrow stem cells has been reported [52, 53].

Besides tha lack of control of long-term transgene regulation and expression in transduced cells, one major problem is the inefficiency of retroviral gene transfer into non-dividing cells like stem cells [54]. Efficient transduction with murine retroviral vectors is achieved only after stem cells are released from quiescence by stimulation with growth factors in culture, which in turn leads to cell differentiation and probably loss of self-renewal and multilineage differentiation capacity [55]. Recently, besides new strategies for efficient and stable retroviral transduction like optimized transduction conditions [56-58], the use of new vector generations like replication-deficient lentiviral-based vectors is emerging. Lentiviral HIV-based vector systems can integrate stably into the host genome in dividing and non-dividing cells, and have been shown to be useful for in vivo and ex vivo gene delivery [59, 60].

Possible implications in gynaecologic oncology

High dose chemotherapy combined with autologous bone marrow or peripheral blood hematopoietic stem cell transplantation has been used with increasing frequency to treat patients with advanced breast cancer and, less frequently, in ovarian cancer [61]. However, most remissions are transient [62]. The major cause for treatment failure is recurrent malignancy, presumably related to incomplete irradication of the disease or reinfusion of contaminated bone marrow or peripheral blood stem cells. Sensitive immunocytochemical and molecular diagnostic techniques have identified occult tumor cells in 60-80% of histologically normal bone

marrow harvests and in 10-80% of peripheral blood progenitor cell collections obtained from patients with stage IV breast cancer undergoing high-dose chemotherapy [63], but its prognostic significance remains unclear [64]. A variety of methods have been used to decrease tumor cell content from collections of hematopoietic progenitor cells (purging or selection). The autologous stem cell graft might however still contain tumor cells after these procedures [65].

Allogeneic stem cell transplantation has also been used in breast cancer [66]. It has two advantages compared to autologous transplantation: First, it avoids tumor cell contamination of the graft, and second, cytotoxic T lymphocytes and NK cells from the donor can lyse tumor cells of the recipient. Accordingly, recent evidence shows that graft-versus-host disease is associated with tumor regression in the recipient, suggesting a clinically effective graft-versus-tumor effect in breast cancer [67]. Allogeneic stem cell transplants might therefore provide an additional benefit regarding the risk of tumor recurrence. Nevertheless, this treatment with allogeneic grafts is associated with additional risk of graft-versus-host disease and related infections as compared to autologous transplants. Therefore, even if only HLA-identical related donors are used, it should at this stage only be performed in the context of clinical trials until the clinical benefit regarding progression-free survival has clearly been demonstrated.

Umbilical cord blood could theoretically also serve as a source for stem cell transplantation for the treatment of solid tumors including breast and ovarian cancer. Autologous cord blood stem cells will probably not be used for this purpose for the next two decades because the first cord blood banks were set up only ten years ago and breast cancer is rare before 30 years of age. Allogeneic cord blood stem cells from related or unrelated HLA-matched donors however might theoretically be used in the not too distant future, if (1) allogeneic transplants prove beneficial for the treatment of breast cancer, and (2) if cord blood provides a clinical advantage over bone marrow or peripheral blood as a stem cell source regarding the risk for graft-versus-host disease and the benefit from graft-versus-tumor effect, and (3) if ex vivo expansion of the limited amount of stem cells available from cord blood becomes feasible. In addition, as significant advances in gene transfer to hematopoietic stem cells are currently achieved [58], cord blood stem cells might in the future be used for antitumoral gene therapy.

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