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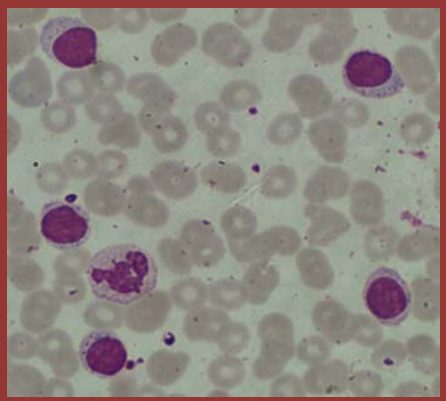

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Editorial

Bone marrow transplantation in Asia – a historical perspective

HWW Goonasekera[1]

The healing power of bone marrow (BM) had even been recognized in Greek mythology; which mention the use of bone marrow extracts from animals to treat injured warriors. In modern medical literature the earliest reports of BM for therapy records oral administration to a patient with leukaemia in 1896[1] and intravenous infusion (IVI) of small volumes of allogeneic BM in 1939 to a 19-year-old girl with aplastic anaemia (AA) from her sibling.[2] The first successful human bone marrow transplant (BMT) was in 1956 by E. Donnall Thomas and his team in New York who administered BM by IVI following total body irradiation (TBI) to two patients with leukaemia from syngeneic twins;[3] their leukaemia initially went into remission, but recurred by 7 and 12 weeks in both. Although the patients did not survive, it demonstrated that it was possible to induce leukaemia remission by isologous BMT following TBI and irradiation doses which can induce remission but not produce side effects such as radiation sickness were described; which was important in the context of the concerns regarding use of irradiation to treat human disease present at that time.

The basic foundations for modern day BMT were based on the seminal experiments carried out by Jacobson *et al.* in the middle of the last century demonstrated that lethally irradiated mice were able to survive if the spleen, or even parts of liver, head and limbs were shielded by lead.[4,5] Other animal model experiments followed which also showed that irradiation followed by marrow infusion resulted in survival, especially if the marrow was derived from mice of similar strain[6] or autologous marrow was used[7] and these and other experiments proved the hypothesis that a cellular factor instead of a humoral factor was responsible for this protective effect[8]; including the experiment which showed presence of donor cells in a recipient mice tagged with a chromosome marker.[9] The main turning point in determining the success of BMT was the discovery of the human leucocyte antigens (HLA) initially described by the French scientist Jean Dausset in 1958[10] and later by others.[8]

E. Donnall Thomas based on his own experimental findings of allogeneic BMT using outbred dogs and knowledge of the HLA system; carried out the first allogeneic transplant for leukaemia[11] and allogeneic transplantation for aplastic anaemia by early 1970s.[12] The first successful matched unrelated donor (MUD) transplant was reported by Thomas *et al.* in 1980.[13] E. Donnall Thomas; who is regarded as the father of BMT; for his discoveries in cell transplantation for treatment of human disease and Joseph E. Murray (who performed the first successful kidney transplant in 1954) were awarded the Nobel Prize in Physiology or Medicine in 1990.[14] For their work on the discovery of the HLA system the Nobel Prize in Physiology or Medicine was awarded to Jean Dausset with Baruj Benacerraf and George D. Snell in 1980.

Transplantation extended to conditions other than leukaemia and the first transplant for immunedeficiency was performed in 1967 on a 5-month-old male child diagnosed with severe combined immunodeficiency, from his eight-year-old sister; both peripheral blood and bone marrow were infused intraperitoneally and the patient had survived.[8,15] The first successful use of cryopreserved autologous marrow to treat lymphomas were reported in 1978[16] and BMT using syngeneic twins for chronic myelogenous leukaemia (CML) with successful eradication of the Philadelphia positive clone was reported in 1979.[17] Successful HLA-matched allogeneic BMT for Thalassaemia[18] and Sickle cell anaemia[19] were reported in July 1982 and June 1988 respectively.

By early 1990s, BMT became established therapy for malignant and non-malignant haematological conditions, immunodeficiency disorders and solid

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tumors.[20] In April 1977, Dr. Thomas *et al.* reported BMT and post BMT sequelae of 100 patients with acute leukaemia describing the complications of BMT and recommended early BMT in acute leukaemia for those who have a HLA matched sibling donor.[21]

From the outset, the main factors for successful outcomes following BMT were graft-versus-host disease (GVHD), graft rejection, relapse, infections and HLA matched donors.[8,11,15,21] Cyclosporin A to prevent GVHD was first described by Powles *et al.* in 1980[22] and prevention of GVHD using a combination of cyclosporine with methotrexate was reported in 1986.[23] Additionally in 1980s T-cell depleted donor marrow to prevent GVHD was described.[24] Use of donor lymphocyte infusion (DLI) were initially described in relapsed patients with CML in 1990[25] and further modifications to improve the efficacy of DLI is being explored.[26] Use of recombinant granulocyte-macrophage colony-stimulating factors to reduce morbidity and mortality following transplantation was initially described in late 1980s and its effectiveness in lymphoid malignancies were proved by Nemunaitis *et al.* in 1991.[27] As allogeneic BMT transplants became more widely used donor registries were established to find a suitable donor; the Anthony Nolan trust in London became the first registry of unrelated bone marrow donors, established by the mother whose son had Wiskott-Aldrich syndrome.[28] By the year 2013, over 25,000 allogenic BMTs were being performed worldwide and marrow donor programs and marrow transplant registries having been established with over 20 million registered volunteer unrelated donors.[29]

In the latter part of the last century advances made in genetic technology rapidly became incorporated into the field of BMT enabling better transplant outcomes. Shifting of HLA typing from serological to molecular methods ensured less donor mismatch and a better chance of marrow engraftment.[30] In 1988 minimal residual disease (MRD) monitoring using polymerase chain reaction technology in place of conventional cytogenetics was reported and with further advances in technology it was possible to quantitate MRD in CML using real-time quantitative PCR by late 1990s and it soon became used in other haematological malignancies.[31]

In 1981 Goldman *et al.* described successful haematopoietic reconstitution following transfusion of autologous blood cells collected from CML patients prior to chemotherapy and TBI[32] and by late 1980s, the advantages of peripheral blood derived haematopoietic stem cells (PB-HSC) over BM derived stem cells were recognized and autologous transplants using PB-HSC started being reported.[33,34] In 1981 Gluckman *et al.* published the first report of umbilical cord blood transplantation (CBT) in a boy with Fanconi's anaemia from his sibling sisters' cryopreserved cord blood.[35] Since then by 2009 over 20,000 CBTs have been done, with establishment of cord blood banks.[36]

Bone marrow transplantation (BMT) has finally taken firm roots in Sri Lanka with starting off of BMT programmes in two leading private hospitals and plans are underway to set up BMT units in the main paediatric hospital, Lady Ridgway Hospital for Children and the leading cancer care centre, Cancer Institute, Maharagama; which are two leading Government Hospitals, in the near feature. In this context, Dr. Rajkumar's leading article which appears in this issue of the *Sri Lanka Journal of Haematology* is timely since he eloquently describes the background and factors pertaining to BMT in the leading article titled "Setting up a blood and marrow transplant (BMT) program in south Asia: Rewards and challenges". Dr. Kumar's article also includes details pertaining to BMT programmes in our region which will be useful for the Haematologists who are currently in the process of setting up of BMT programmes in the above mentioned hospitals as well as the Haematology trainees who will have to take up the challenge of carrying these initiatives forward once they qualify and become Consultant Haematologists in local BMT units.

Furthermore, Dr. Agarwal's perspective on treatment for leukaemia will be read with enthusiasm especially by the Haematologists who are actively involved in treating haemafo-oncology patients.

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Leading article

Setting up a blood and marrow transplant (BMT) program in South Asia: Rewards and Challenges

Rajat Kumar[1]

Key words: blood and bone marrow transplant, haematopoietic stem cell transplant, aplastic anaemia, haploidentical transplants

Introduction

Blood and marrow transplantation (BMT) or haematopoietic stem cell transplantation (HSCT) is a life-saving procedure for a number of malignant and non-malignant life threatening diseases.[1,2] More than a million procedures have been completed worldwide, and the annual transplant rate is close to 70,000 per annum with approximately 45% being allogeneic.[3] The procedure itself has many technical variations according to the primary disease, age of the patient, facilities available and experience of the center. BMT may be autologous or allogeneic. When the patient's own cryopreserved haematopoietic stem cells are used to rescue bone marrow after high dose chemotherapy, the procedure is termed as autologous transplantation. Allogeneic BMT involves the transplantation of haematopoietic stem cells derived from another individual, ideally a human leucocyte antigen (HLA) identical sibling, into the patient. In this article, allogeneic HSCT will be discussed. As aplastic anaemia and thalassaemia major are common in South Asia, only these indications will be highlighted.

Indications for HSCT

The indications for allogeneic (allo) haematopoietic stem cell transplantation in haematological disorders can be conveniently divided into two groups (Table 1): (a) Malignant disorders – like leukaemias, myelodysplastic syndromes and lymphomas. In all these indications, the cure is by a combined effect of the high doses of chemotherapy or radiation therapy, and a graft versus tumor effect by the transfused donor cells. The transfused donor cells also "rescue" the bone marrow from the effects of chemotherapy. (b) Non-malignant diseases – like aplastic anaemia, thalassaemia, Gaucher disease, etc. In these conditions the abnormal marrow is replaced by the healthy donor stem cells.

Allogeneic HSCT

Stem cell source

The three sources of stem cells used in HSCT are the bone marrow, peripheral blood and cord blood. The three sources differ in the stem cell content, composition and state of activation of immune cells. Quantitatively, peripheral blood represents the richest stem cell source and cord blood the poorest stem cell source. Peripheral blood contains more lymphocytes than the other two sources. The most rapid engraftment is observed with peripheral blood transplants and the slowest with cord blood transplants. The risk of developing graft-versus-host disease (GVHD) also varies with the source of stem cells. Peripheral blood stem cells (PBSC), which contain more T lymphocytes than marrow does, increase the incidence and severity of chronic GVHD compared with bone marrow, while cord blood transplants have a lower risk of GVHD.[4,5] The trends in stem cell source for transplantation are changing. A report from the European Group for Blood and Marrow transplantation (EBMT) showed that in 2011 a total of 35,660 HSCT were reported with 41% allogeneic and 59% autologous by 651 centers in 45 countries.[6] Peripheral blood stem cells were used as a stem cell source in 99% of autologous and 73% allogeneic HSCT. Bone marrow was used as a stem cell source in allogeneic transplantation, primarily for nonmalignant disorders. Cord blood was used for 6% of allogeneic HSCT, mainly from unrelated donors and no autologous cord blood was used.[6]

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Table 1. Indications for allogeneic stem cell transplantation

Malignant disorders	Non malignant disorders
Acute myeloid leukaemia	Thalassaemia major
Acute lymphoblastic leukaemia	Aplastic anaemia
Chronic lymphocytic leukaemia	Fanconi's anaemia
Chronic myeloid leukaemia	Myeloproliferative disorders
Myelodysplastic syndromes	Sickle cell anaemia
Non-Hodgkin lymphoma	Paroxysmal nocturnal haemoglobinuria
Hodgkin disease	Severe combined immunodeficiency
Multiple myeloma (mainly autologous)	Inborn errors of metabolism

Donor requirement for allogeneic HSCT

HLA-identical sibling

For an allogeneic BMT, an HLA identical sibling is the ideal donor. A sibling who is identical in the HLA-A, B, DR loci is considered HLA identical implying a 6/6 match. In spite of HLA identity, there are always variations in the minor histocompatibility loci. These antigenic differences lead to graft rejection or graft versus host disease unless immunosuppression is used. It is also possible to have a successful transplant using a partially matched sibling as a donor, or an unrelated HLA identical donor, but the complications of GVHD and graft rejection increase.

Unrelated donors

For unrelated transplants, the HLA-C and HLA-DQ loci are also tested and a 10/10 match is ideal. For unrelated umbilical cord blood transplants, a 6/6 or even a 4/6 match is acceptable as the cord blood cells are immunologically naïve and the risk of GVHD is less. With improvements in HLA typing at the molecular level, results of unrelated transplants are often equivalent to matched sibling transplants and at times may be preferred. The massive increase of unrelated donor registries has increased the likelihood of finding a well-matched unrelated donor in addition, there is increasing evidence that the well-matched donor in certain situations might be preferable to a sibling donor, for example, in the situation of an older male patient with the choice between an older female sibling donor and a young well-matched unrelated male donor.

Haploidentical related donors

Mendelian genetics dictate that each biological parent and each biological child of a patient is HLA-haploidentical, each sibling, half-sibling, aunt or uncle has a 50% likelihood of being HLA-haploidentical.[3] Thus a haploidentical (haplo) donor can be found for nearly every patient requiring an allo-HSCT. Moreover, this is likely to be faster than a search for an unrelated donor.

Choice of donor

The best outcomes for allo-HSCT are with a donor who is a HLA-matched sibling. There is 25% chance of each sibling being HLA-identical, and with the small family sizes, only 30% chance that a patient will find a matched sibling donor.

The next alternative is a matched unrelated donor (MUD)[7]. The unrelated donor pool has expanded to more than 26 million donors worldwide, with an increased number of unrelated allo-HSCTs being performed. With improvement in protocols, outcomes with matched unrelated donor transplants rival those after matched sibling transplants[3]. Suitable matched unrelated donors (MUD) are found for 60% to 80% of white Caucasians but only for 10% of ethnic minorities.

For those who do not have HLA-matched related or unrelated donors, the options are (a) mismatched unrelated donor (MMURD) (b) unrelated donor umbilical cord blood or (c) HLA haploidentical, related donors.[3,7]

Conditioning procedure

Myeloablative conditioning. The standard preparatory regimens given prior to HSCT are myeloablative. Patients receive extremely high doses of chemotherapy or radiotherapy or both. The aim is threefold: (a) Eradication of malignant cells or, in cases of genetic disorders, it is eradication of the abnormal clone of cells, (b) Suppression of the immune system of the host (recipient) so that the allograft is not rejected, and (c) Clearing a "physical space" to allow adequate growth of the donor stem cells. The conditioning, which is myeloablative, is also toxic to various organs like the liver, lungs, kidneys, gastrointestinal tract and reproductive system.

Non-myeloablative or reduced-intensity conditioning. The association of GVHD with diminished relapse rates following allogeneic HSCT, together with the dramatic responses sometimes seen following donor lymphocyte infusions demonstrates the potential of the human immune system to eradicate haematological malignancies. The curative potential of allogeneic BMT is mediated in part by an immune mediated graft-versus-tumor effect. This has prompted some workers to focus on the use of donor T cells to eradicate both non-malignant and malignant cells of host origin, without the use of myeloablative conditioning regimens. This reduced-intensity conditioning (RIC) aims to suppress the immunity of the recipient sufficiently to allow allogeneic engraftment, without destroying the recipient's marrow, with lower regimen related toxicity. This represents an important step in capitalizing on the allogeneic graft-versus-tumor effect.[8]

Technical aspects of allogeneic transplantation

Bone marrow transplantation

The actual bone marrow transplant is not complicated. The donor's marrow is harvested by repeated aspiration from the posterior iliac crests, under general or spinal anaesthesia. The marrow is collected in a bag with anticoagulant. The number of marrow cells or total nucleated cells (TNC) required for successful engraftment is estimated to be at least 1 to 3 x 10^8 per kg of recipient's body weight. Bone marrow is transfused through the veins and the donor marrow cells home into the recipient's marrow space and start engrafting. Engraftment is considered established when the peripheral neutrophil count reaches 0.5 x 10^9/L on 3 successive days.

Peripheral blood stem cell transplantation (PBSCT)

It is well known that the peripheral blood contains a small percent of circulating stem cells, approximately 0.1%. This number can be increased by administration of colony stimulating factors, like Granulocyte-colony stimulating factor(G-CSF), which mobilize stem cells from the bone marrow. For allogeneic donors, administration of G-CSF for 4 to 5 days results in high circulating stem cells which can be collected by a cell separator. The procedure requires venous access and takes about three to four hours. The donor need not be admitted, does not require anesthesia and is spared the pain of marrow aspiration. Haematopoietic reconstitution is more rapid and predictable when PBSCs are used for transplantation. This translates in reduced duration of neutropenia, fewer platelet transfusions, and shorter hospital stay (Table 2). Immune reconstitution may be better with PBSCT as there are more lymphocytes in the graft as compared to marrow.

Cord blood stem cell transplantation

Placental blood, which is routinely discarded in clinical practice, is potentially a vast supply of allogeneic foetal haematopoietic stem cells. Cord blood (CB) stem cells have distinctive proliferative advantages which include an (a) enriched proportion of immature stem cells, (b) higher clonogenic growth advantage, (c) increased cell cycle rate, (d) autocrine growth factors production and (e) increased telomere length.

The main limitation of cord blood transplants (CBT) is the limited number of nucleated cells available in a unit. As compared to bone marrow transplantation, the time for engraftment in cord blood transplantation is much longer, taking a month for neutrophilic engraftment and more than fifty days for platelet engraftment. There is also a higher incidence of non-engraftment. The nucleated cell dose available in a cord blood unit is critical, being 1 log less than in a bone marrow transplant. The minimum recommended dose for CBT is 2.0 to 2.5 x 10^7 nucleated cells /kg for a successful outcome and at least a 4/6 HLA match. The main advantage of CBT is a lower incidence and severity of GVHD.

Table 2. The advantages and disadvantages of PBSCT

	Advantages	Disadvantages
Recipient	1. Faster neutrophil recovery 2. Faster platelet recovery 3. Faster immunologic recovery 4. Less IV antibiotics 5. Shorter hospitalization 6. Lower cost 7. More graft versus tumor effect (?)	Higher incidence of chronic GVHD
Donor	1. No general anesthesia 2. No marrow harvest 3. No hospitalization	1. Venepuncture or central line 2. Side effects from growth stimulating factors, or citrate used for PBSC harvest

PBSCT – Peripheral blood stem cell transplantation, IV – Intravenous, GVHD – Graft-versus-host-disease, PBSC – Peripheral blood stem cell

This allows a 1 to 2 HLA antigen mismatch even in unrelated CBT. More than 600,000 cord blood units have been stored worldwide and over 30,000 CBT have been performed, mainly in the unrelated setting.[9]

The main problem in doing CBT in adults is the limited number of nucleated cells/CD34+ cells in a cord blood collection relative to the weight of an adult. Different strategies are being investigated for this; these include (a) multiunit cord blood transplantation, (b) ex-vivo expansion of CB haematopoietic stem cells (c) nonmyeloablative preparative regimen to reduce the conditioning toxicity[5,9].

Haploidentical related transplantation

In the past, results of haplo-HSCT were poor due to high incidence of graft failure or GVHD, due to bi-directional allo-reactivity. This is due to the high frequency of T lymphocytes that recognize major class I or II HLA disparities between the donor and recipient.

In the last two decades, the outcomes of haplo-HSCT have improved and results are comparable to unrelated HSCT for malignant disease indications. There are three main approaches to control graft failure or GVHD. (1) The megadose HSCT approach using PBSCs positively selected for CD34+ cells, developed in Italy. The average dose of cells is >10 x 10^6 CD34+ cells/kg body weight of recipient.[10] (2) The GIAC protocol used in China, comprising of G-CSF stimulation of donor; Intensified immunosuppression using post-transplant cyclosporine, mycophenolatemofetil and short course methotrexate; ATG added to conditioning to aid engraftment and help prevent GVHD; and combination of PBSC and BM allografts[11]. (3) High dose, post transplantation cyclophosphamide[12].

Post-transplantation cyclophosphamide was first developed in animal models and finally translated into clinical practice by groups at Johns Hopkins and Fred Hutchinson Cancer Research Center.[13] It was shown that cyclophosphamide is nontoxic to haematopoietic stem cells because of high expression of the detoxifying enzyme aldehyde dehydrogenase. Moreover, cyclophosphamide is selectively toxic to dividing cells.

With post-transplant cyclophosphamide, the first step is selective killing of proliferating alloantigen-stimulated T cells. As replicating T cells are uniquely sensitive to cyclophosphamide, both anti-host and anti-donor T cells are selectively destroyed. The quiescent progenitor cells and memory T cells in the graft are not affected. Persistence of donor non-alloreactive T cells can provide the transplant

recipient with donor-derived immunity to fight infections. The second step is the development of peripheral tolerance. The third step is deletion of donor stem cell derived anti-host T cells in the thymus.[12] Cylophosphamide is usually given on days 3 and 4 post stem cell infusion at doses of 50mg/kg on each day.[14]

This protocol is used widely in the United States and many other countries, including India. Number of studies have used this protocol in myeloablative as well as reduced intensity conditioning, with BM or PBSC as stem cell sources.[13,14] This approach has shown acceptable incidence of GVHD and low non relapse mortality. When myeloablative conditioning was used, the relapse rate was lower than with RIC and results comparable to those with MUD or cord blood transplant. It is the most attractive protocol for haplo-HSCT in developing countries, as no special equipment is needed for stem cell manipulation, there is no need for expensive medications, the cost of cyclophosphamide is low and it is given by simple intravenous administration.

Supportive care

Protective isolation. After transplantation of the marrow, it takes about two to three weeks before engraftment occurs, that is the time when the stem cells start producing adequate number of neutrophils, platelets and erythrocytes. During this period very intensive support is required. Ideally a high-efficiency particulate arrestance (HEPA) filtered unit should be available for hospitalization for transplantation. It is emphasized that the risk of infections lasts for almost a year in allogeneic HSCT. Some centers have reported carrying out stem cell transplants without protective isolation, or even in outpatient setting, without increase in morbidity or mortality.[15] This is only feasible if the home offers a clean environment, the patient can be monitored closely and admitted immediately, if required. At the All India Institute of Medical Sciences (AIIMS), the department of haematology recently published its experience of performing 40 consecutive allogeneic transplants from July 2004 to November 2007 in single non-HEPA filter rooms for a variety of indications. Source of stem cells was peripheral blood in 33, bone marrow in six and combined in one. The indications were severe aplastic anemia-18, chronic myeloid leukaemia (CML)-7, acute myeloid leukaemia (AML)-7, acute lymphoblastic leukaemia-2, myelodysplastic syndrome-2 and thalassaemia major-4. The median age was 19 years (range 2.2-46) with 29 male and 11 female participants. The 30-day mortality was nil, and 100-day mortality was 1 (2.5%). This experience suggests that allogeneic HSCT can be safely performed in non-HEPA filter rooms in India.[16] Updated results confirm that this approach is effective in high risk patients.[17]

Venous access. The transplant process typically involves the use of a long term, silastic, multilumen, flexible catheter for chemotherapy administration, infusion of stem cells and supportive care management including frequent blood sampling, intravenous antibiotics, blood components and parenteral nutrition.[18]

Infections. Infection remains an important cause of morbidity and mortality after BMT, with bacterial, fungal infections and viral infections being the predominant cause.[19] The EBMT analyzed a large homogeneous group of 14,403 patients transplanted for early leukaemia from an HLA-identical sibling and reported to the EBMT from 1980 to 2001. Of the 597 deaths with infection as the primary cause of death, 217 (36%), were attributed to bacteria, 183 (31%) to viruses, 166 (28%) to fungi and 31 (5%) to parasites. The cumulative incidence of deaths with infection at 5 years was 5% with a cumulative incidence of 1.8% attributed to bacteria, 1.6% to viruses, 1.4% to fungi and 0.3% to parasites[19]. During the early neutropenic period, bacterial and fungal infections predominate while viral infections are frequent after engraftment when the cell mediated immunity is impaired, the most important viruses being cytomegalo virus, herpes simplex virus, and varicella zoster. Bacterial infections with encapsulated organisms again predominate after three to six months of engraftment, akin to the condition in post splenectomised patients antimicrobials should be administered after establishing the cause of infection, but in practice an aetiological agent is often not identified. During the neutropenic phase, early institution of empirical antibiotics to cover gram-negative and gram-positive bacteria, with addition of antifungal drugs like amphotericin or voriconazole if fever persists, is practiced in most centers in India[16].

Blood component support. After conditioning therapy, patients require multiple red cell and platelet transfusions during the 2-4 week period

of pancytopenia, till engraftment occurs. Patients are profoundly immunosuppressed and at risk of developing transfusion associated-graft versus host disease (TA-GVHD) after receiving cellular blood products. To prevent this, all cellular blood products should be irradiated prior to transfusion, to inactivate the donor lymphocytes. Hence a blood irradiator is essential for any allogeneic BMT center.

Haematopoietic growth factors. Haematopoietic colony stimulating factors like G-CSF are often administered to patients after infusion of stem cells in order to reduce the duration of neutropenia. More recently, studies have shown that even without use of these factors there is no adverse impact on outcome, and many centers use them only in cases with delayed engraftment.

Toxicity related to conditioning. The conventional myeloablative therapy given before infusion of bone marrow causes organ toxicity, in addition to myelotoxicity. These are: (a) veno-occlusive disease (VOD) of the liver, more accurately termed as "sinusoidal obstruction syndrome". It is characterized by (i) jaundice (ii) hepatomegaly and right upper quadrant pain (iii) ascites or (iv) unexplained weight pain, (b) haemorrhagic cystitis characterized by the presence of haematuria, dysuria, and urinary frequency in a patient with sterile urine, (c) seizures usually drug induced, (d) pulmonary complications which can be infectious or non-infectious, and (d) skin and mucosal changes like alopecia, nail changes and oral mucositis.

Failure of engraftment

Failure to engraft after HSCT (graft dysfunction) or to sustain engraftment (graft rejection) is a formidable complication due to many possible factors. These include inadequate stem cell numbers, infections, graft-versus-host disease and immunological mediated processes. The stem cell graft may get rejected by functional host lymphocytes which survive the conditioning regimen. Fortunately, this complication is uncommon. Multiple treatment alternatives have been explored including haematopoietic growth factors, additional infusions of stem cells alone, with augmented immunosuppression or with additional cytotoxic therapy. The incidence is higher in unrelated donor transplantation and whenever there is presence of any HLA mismatch. Depleting the graft of T cells also increases graft rejection.

Graft-versus-host-disease (GVHD)

In allogeneic HSCT patients, a unique complication occurs: GVHD. There are two types of GVHD, acute and chronic.[2] Acute GVHD: This occurs within the first 100 days after transplant. It classically affects three tissues, namely the skin, gut and liver and may be accompanied by fever. The severity can be graded according to the extent of skin involvement, degree of hyperbilirubinemia and severity of diarrhoea.[2] Chronic GVHD: This usually develops later than 100 days after transplant and often follows acute GVHD but may also develop de novo. It is classified as limited or extensive chronic GVHD. Clinically it resembles autoimmune disorders like scleroderma with skin rash, sicca complex, sclerosing bronchiolitis and hepatic dysfunction. The mortality varies from 20% to 40%. Management is with immunosuppressive agents like cyclosporine, prednisolone, tacrolimus, mycophenolate, sirolimus, methotrexate and cyclophosphamide in various combinations. After a year or more, many patients develop self-tolerance and these drugs can be tapered off. GVHD is more common in older patients and those with one or more HLA mismatches or unrelated HLA identical transplants. It is mainly for this reason that elderly patients do not do well with allogeneic BMT due to severe GVHD. With the use of PBSC, the time limits are not so well defined and acute GVHD may occur later while classical chronic GVHD may occur earlier.

Tumor relapse

A successful BMT does not always mean that the primary disease is cured. A certain number of patients will relapse from the original malignancy, as the tumor cells survive the chemo/radiotherapy and graft versus tumor effect. Relapses are higher if the HSCT is performed when the disease is not in remission, or at an advanced stage, or is aggressive.

Patients with haematological malignancies who relapse after allogeneic bone marrow transplantation can be treated by infusing lymphocytes from the original stem cell donor. Donor lymphocyte infusion (DLI) induces complete remissions in the majority of patients with CML in early-stage relapse and in less than 30% of patients with relapsed acute leukemia, myelodysplasia, and multiple myeloma.

Requirements for BMT unit

The complexity of the transplantation procedure highlights the need for adequate infrastructure, experience and teamwork, to manage patients who can potentially have multiple complications. A multispecialty approach is needed. An efficient blood bank and transfusion center with facilities for blood component therapy is critical. Blood irradiation facilities are essential. As infections are common in early and late phases, facilities for diagnosis and management of common and rare bacterial, fungal, viral and parasitic infections are needed. For organ toxicity and GVHD management, subspecialties of gastroenterology, pulmonology, hepatology, cardiology, dermatology, ophthalmology, histopathology and others are required. Documentation of all cases and prospective registries are needed. Guidance may be obtained from the Center for International Blood and Marrow Transplant Research (CIBMTR) or the EBMT. As a general guide, a center should be capable of successfully treating patients of AML, before embarking on a BMT program.

Indications for allogeneic transplants

In recent years, evidence based guidelines have been formulated for indications in haematologic disorders. These may change with improvements in non-transplant therapy. As an example, allo-HSCT was the first line treatment for patients of CML who were eligible and had a donor, but with the availability of tyrosine kinase inhibitors, the indications of transplant have reduced radically.

Aplastic anemia

Severe aplastic anemia (SAA) is potentially curable with allo-HSCT, the only limiting factor being the transplant related morbidity and mortality. Guidelines suggest that in young patients with an HLA matched sibling donor, allo-HSCT should be first line therapy, as the complications are much less (Table 3). In those who are older than 50 years, immunosuppression should be tried first.[20] The source of stem cells is also controversial. A recent study showed better outcome with bone marrow versus peripheral blood. This was mainly due to a higher GVHD in the PBSCT group.[21] This study analyzed the outcome of 692 patients with severe aplasticanemia receiving transplants from HLA-matched siblings. A total of 134 grafts were PBSC grafts, and 558 were bone marrow grafts. Rates of haematopoietic recovery and grades 2 to 4 chronic GVHD were similar after PBSC and bone marrow transplantations regardless of age at transplantation. In patients older than 20 years, chronic GVHD and overall mortality rates were similar after PBSC and marrow transplantations. In patients younger than 20 years, rates of chronic GVHD (relative risk [RR] 2.82; *P*=.002) and overall mortality (RR 2.04; *P*=.024) were higher after transplantation of PBSCs than after transplantation of bone marrow. These authors concluded that bone marrow grafts are preferred to PBSC grafts in young patients undergoing HLA-matched sibling-donor transplantation for SAA.[21] A similar conclusion was reached by another EBMT study.[22]

This view is not accepted by many experts in the developing world. Patients with aplastic anemia who come for transplantation in developing countries are often multi-transfused and the blood products they receive are usually not leuco-depleted. They are therefore alloimmunised and have a high risk of graft rejection. PBSC transplant reduces the chances of graft rejection due to a higher stem cell dose and the higher T cell content. Moreover, many patients are infected prior to coming for transplant and a PBSC graft source has the advantage of an earlier engraftment as well as immune reconstitution. The transplant centers at AIIMS New Delhi and Christian Medical College (CMC) Vellore routinely use PBSC as a preferred source for allo-HSCT in aplastic anemia.[23,24] The success rates of 70-80% survival suggest that in the Indian context, PBSC may be a preferred source of stem cells in the high risk patients seen in India. A recent analysis of 2374 HLA identical sibling transplants in SAA across different economic zones, confirmed the observation that overall, BM is a better source than PBSC. However, there was no difference in overall survival between the two graft sources in upper-middle-income and lower-middle and lower-income countries, hence PBSC may be an acceptable alternative in countries with limited resources when treating patients at high risk of graft failure and infective complications.[25] The use of haploidentical transplants is considered experimental, but in future it may become a preferred source for those without sibling donors, if the problem of graft rejection can be overcome.[20]

Table 3. BMT in aplastic anaemia

Indication	Treatment recommendation	Comments
Severe aplastic anemia	If HLA identical sibling available. (a) Age < 50 yr: BMT 1st line treatment. (b) Age >50yr: BMT as 2nd line treatment if immunosuppression fails in 3-4 months. If only HLA identical unrelated donor available. Age < 50 years: BMT if Immunosuppression fails.	The indications of age may be relaxed for: (a) Patients who are infected and would not tolerate immunosuppression or (b) very severe aplastic anaemia

BMT – Blood and marrow transplantation, HLA – Human leucocyte antigen

Thalassaemia major

A major indication for allo-HSCT in India is thalassaemia major. This disease is potentially curable with an allogeneic transplantation. The results are excellent if the transplantation is done prior to the complications of iron overload, transfusion complications and alloimmunisation. Results from Pesaro, Italy, suggest more than 85% disease free survival for patients transplanted early, in Pesaro Class 1.[26] In India, similar results have been attained at CMC, Vellore.[27] When a child is diagnosed with thalassaemia major, treatment should be started with optimal blood transfusion and iron chelation instituted before there is a significant rise in ferritin levels. All siblings should be typed for an HLA identical match. If a match is available, the child should be referred to a transplant center. An allo-HSCT should be performed as soon as feasible. For convenience of nursing and post-transplant care, allo-HSCT in India is generally performed after the child is more than 3 years of age.

Economic aspects

Haematopoietic stem cell transplantation requires significant infrastructure. In the first report by the Worldwide Network for Blood and Marrow Transplantation, it was concluded that no HSCTs were performed in countries with less than US $680 gross national income (GNI) per capita[28]. Transplant activity is concentrated in countries with higher governmental health care expenditures, higher GNI per capita, and higher team density. In countries with limited resources, HSCT is preferentially restricted to allogeneic transplants with stem cells from family donors for non-malignant indications or chronic leukaemias[29].

In India, the cost of an allogeneic HSCT varies as per the indication and the type of hospital (Government funded or private). The cost of an allogeneic transplant in AIIMS, New Delhi, varies between US $6000 to 12,000. The cost of medication and monitoring after engraftment is additional. In private corporate hospitals, the cost varies between US $15,000 to 30,000 for initial hospitalization. The variation in cost depends on the conditioning regimen, complications and the use of anti-infectives and supportive drugs. Thus the use of anti-thymocyte globulin would significantly increase the cost substantially.

In terms of unrelated transplants, the cost of the graft source may have a major impact in choice of graft in developing countries. The approximate cost of an unrelated umbilical cord blood unit is US $30,000 while the cost for a matched unrelated donor stem cells varies between US $12,000 to 30,000 based on the registry. From a purely financial aspect, haplo-identical donor would be cost effective, provided the outcome was similar to unrelated donor transplants.

Summary

Allogeneic HSCT should be offered to patients where the benefits outweigh the risks. Non-

transplant therapy should be compared to HSCT, before making any recommendations. Counseling of the patient and patient preference is extremely important, as HSCT involves considerable expense, often prolonged morbidity and potential fatality. In developing countries such as in South Asia, where the cost is usually borne by the patient and family, economic factors need consideration. In general, early transplant offers better results than HSCT performed in advanced disease, but in those disorders where non-transplant therapy offers similar outcomes, HSCT is offered when there is failure of alternative therapy. Aplastic anemia and thalassaemia are the most common non-malignant indications for transplant in South Asia. In haematological malignancies, cytogenetic and molecular prognostic markers usually guide the timing of transplantation. Potential transplant candidates should be referred early to a transplant center where facilities for assessment are available. For more detailed disease specific analysis, the original articles should be reviewed.

Authorship

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Perspective

Treatment of elderly acute myeloblastic leukaemia with azacitidine after failure of decitabine

MB Agarwal[1]

Key words: acute myeloblastic leukaemia, azacitidine, decitabine

Summary

A 72-year-old man was seen with acute myeloblastic leukaemia (AML) after negligible response to four cycles of decitabine. Patient was treated with subcutaneous 5-azacitidine 75 mg/m^2 daily x 7 days/cycle. Patient achieved complete remission with incomplete recovery of blood counts (CRi) after 6 cycles and has been on maintenance cycles with the same schedule for another 8 months so far. There is very little data to support use of 5-azacitidine in elderly AML after failure of decitabine and hence this report.

Case report

A 72-year-old man was seen for AML. He had symptoms of weakness and episodic fever. There was easy bruising and one episode of epistaxis. Earlier, at another centre, he was diagnosed as AML 6 months earlier. He had presented with vague systemic symptoms, pancytopenia with marrow showing 30% blasts. During those 6 months, he had received 4 cycles of decitabine 20 mg/m^2 daily x 5 days per cycle as intravenous infusion therapy. Throughout this period, patient was off and on extremely sick due to anaemia and recurrent infections needing repeated hospitalizations and blood component support. Marrow repeated after 2 cycles had shown 28% blasts and the same after 4 cycles had shown 52% blasts. His subsequent treatment was abandoned and he was advised best supportive care.

At presentation to us, patient was sick, febrile with mild hepatosplenomegaly (1 cm each) and purpuric spots all over the body and oral cavity. His haemoglobin (Hb) level was 51 g/L, platelet count 26 x 10^9/L, WBC count 2.8 x 10^9/L with 18% blasts in peripheral blood and 78% blasts in the marrow. There were dysplastic changes in all 3 cell lines. Immunophenotyping confirmed AML with CD13, CD33, CD117, CD34, HLA-DR and cMPO positivity. Cytogenetic studies showed 7-monosomy. Molecular studies showed no evidence of nucleophosmin *(NPM1)* gene mutation or FMS-like tyrosine kinase-3 internal tandem duplication mutations *(FLT3/ITD)* mutation. Patient had performance status of two. He had no comorbidities.

He desired therapy with minimal toxicity over and above best supportive care. However, he had no interest in standard chemotherapy using 3+7 protocol or reduced intensity transplant. In view of this, he was started on subcutaneous (SC) azacitidine 75 mg/m^2 x 7 days every 28 days on outdoor basis. He was also on levofloxacin and voriconazole prophylaxis. The patient had local reactions at injection sites, episodic significant gastrointestinal disturbances and continuous requirement of blood products. However, by the end of third cycle, there was significant decrease in transfusion requirement and peripheral blood counts showed improvement. By the end of 6th cycle, he had a Hb level of 92 g/L, platelet count of 86 x 10^9/L (unsupported), WBC count of 6.4 x 10^9/L with absolute neutrophil count (ANC) of 4.9 x 10^9/L and no blasts in peripheral blood. Marrow examination showed good cellularity, minimal trilineage dysplasia with blasts < 1%. Cytogenetic studies showed persistence of 7-monosomy.

Patient was reluctant to continue further treatment. However, he was convinced and he continued the same treatment with almost no toxicity. By May 2015, he had completed total of 14 cycles

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and he was in complete remission (CR) with Hb level of 124 g/L, platelet count of 136 x 10^9/L, WBC count of 5.6 x 10^9/L with ANC of 2.9 x 10^9/L and no blasts in the peripheral blood. Bone marrow was not repeated.

Discussion

This is an interesting case where a number of lessons could be learnt.

The incidence of AML in patients over 70 years old is > 20 times greater than that observed in younger subjects. Elderly AML differ from young patients in various ways; high incidence of poor prognostic cytogenetic abnormalities, high incidence of therapy-related leukaemia, association with a prior haematological disease, often presence of multi-drug resistance gene expression and high incidence of comorbidities and poor tolerance to chemotherapy.

Overall, there is reluctance in treating elderly AML, especially in the developing world. Achievement of CR improves the quality of life and may also add to the duration of life. Hence, it makes sense to have a therapeutic plan with positive intentions even for such patients.

Although, there is no standard treatment regimen, elderly AML can be treated with one of the following options; standard induction therapy using 3+7 regime consisting of anthracyclin and cytosine arabinoside (Ara-C) with or without reduced intensity bone marrow transplantation, hypomethylating agents (HMA), low-dose cytosine arabinoside (LD-AraC), best supportive care (BSC) and clinical trial.

Standard induction therapy which is best for younger subjects may be the first choice even in elderly AML, however, there is early death of 15% in most of the studies. Development of hypomethylating agents (HMA) has brought out a new ray of hope in this group of AML patients.

Hypomethylating agents were discovered almost 50 years ago. Initially, they were used in high doses for treating AML. Results with 3+7 protocol were superior and hence HMA were almost forgotten. In early part of this century, the interest in HMA for treating myelodysplastic syndrome (MDS) was revived as their use in low dose worked as differentiating agents with good success and minimal toxicity. With accumulation of substantial data, 5-azacitidine received United States Food and Drug Administration (US-FDA) approval for treatment of MDS in 2004 and decitabine received the same in 2006.

Over last few years, HMA were considered as an attractive strategy for treating patients of AML who were otherwise considered unsuitable for 3+7 therapy. Azacitidine was compared with various conventional care regimens (CCR) which included LD-AraC, intensive chemotherapy or supportive care (phase 3 trial) in patients with intermediate-2 and high-risk MDS. Interestingly, 113 patients in this series had blasts between 20% and 29% and therefore, these were cases of AML with low blast counts. Complete remission rates were similar in the two arms (18% vs 16%)[1].

Subsequently, azacitidine vs CCR was studied in elderly AML with any blast count. Azacitidine showed improved median OS (10.4 months vs 6.5 months, p=0.08). This was statistically significant. A pre-planned sensitivity analysis censored for subsequent AML treatment showed a benefit in terms of median OS of 12.1 months vs 6.9 months for azacitidine[2]. Currently, azacitidine has licensed approval from European Medicines Agency (EMA) for AML with 20% - 30% blast cell count.

Decitabine 20 mg/m^2 daily for 5 days per cycle has also been studied against CCR in a phase 3 trial of 485 patients of AML above the age of 65 years. There was a higher response (CR + CRi 17.8% vs 7.8%) and better survival. This reached statistical significance (median OS 7.7 vs 5.0 months)[3]. Currently, decitabine has approval by EMA for patients of 65 years and above with AML who are not considered candidates for standard induction therapy.

Both azacitidine and decitabine have been approved by the US-FDA for AML with 20% to 30% of blasts. Studies have shown that Ten-Elevan-Translocation-2 *(TET2)* and DNA methyltransferase 3A *(DNMT3A)* mutated AMLs benefit from these epigenetic agents[4].

Decitabine has also been used in another more intensified dose schedule of 20 mg/m^2 daily for 10 days in 53 patients with median age of 74 years and the outcome was encouraging[5]. Complete remission rate was 47% and CRIi 17%.

Dombret *et al*.[6] have published the results of international phase 3 study of azacitidine vs conventional care regimens in older patients with newly diagnosed AML with >30% blasts. This is a study of 488 patients over the age of 65 years with newly diagnosed AML having over 30% marrow blasts. Median overall survival (OS) was longer with azacitidine vs CCR i.e. 10.4 months vs 6.5 months. Univariate analysis showed favourable trends for azacitidine compared with CCR across all subgroups. They concluded azacitidine as an important treatment option for this difficult to treat AML population.

Ramos F *et al.*[7] on behalf of European AML investigators have published their observations in using azacitidine as frontline therapy for unfit AML patients. This study includes newly diagnosed unfit patients of AML treated in France, Austria and Italy. European LeukaemiaNet response was achieved in 21.0% of 371 patients. This did not depend on bone marrow blast cell percentage. Median OS was 9.6 months and 40.6% of patients were alive at one year.

Lao Z *et al.*[8] concluded that treatment of azacitidine in elderly subjects with AML leads to fewer hospitalisation days and infective complications but similar survival compared with intensive chemotherapy.

Outcome of patients with AML who have failed treatment with HMA is poor. Median survival is 6 months. There is no established therapy available except allogeneic haematopoietic stem cell transplantation.

The choice between azacitidine and decitabine as the initial treatment of MDS or AML remains in dispute.

Our patient is unique from the angle that azacitidine worked after failure of decitabine. This goes to show that there are subjects where there may be no cross resistance between these two HMA. Recently, we have used azacitidine + lenalidomide after failure of decitabine with good success (unpublished observation). Future probably lies in rationally designed combination therapy in these otherwise difficult to treat patients.

Authorship

Contribution: This is the sole work of Dr. M.B. Agarwal.

Conflict-of-interest disclosure: The author declares no conflict of interest.

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SLJH. 2015; **7**(1): 19-23

Research paper

Hospital admission rate, pattern of lower limb deep vein thrombosis (DVT) and its relationship to acquired risk factors among patients admitted to Colombo South Teaching Hospital (CSTH) during the year 2010

WHE Alwis[1], **N Ranasinghe**[2]

Key words: deep vein thrombosis, risk factors, hospital admission

Abstract

Deep vein thrombosis (DVT) confers considerable mortality and morbidity. However there is hardly any data available on prevalence and pattern of DVT among the Sri Lankan population. The study was carried out with the aim of assessing hospital admission rate, pattern of lower limb DVT (LL-DVT) and its relationship to acquired risk factors among patients admitted to a tertiary care hospital. Total of 34 patients were identified with LL-DVT for the year 2010. Data were collected from bed head tickets of discharged patients, anti-coagulation clinic records and doppler ultrasound scan lists. The hospital admission rate of LL-DVT among the study population was 25 per 100,000 admissions per year. Sixty five percent (n=22) had proximal LL-DVT whereas 21% (n=7) had distal LL-DVT. An acquired risk factor was identified in 68% (n=23). Forty eight percent (n=11) of patients had immobility as an acquired risk factor and 26% (n=6) of patients had multiple risk factors. Eight patients (47%) with proximal LL-DVT had non transient risk factors compared to none of the patients with distal LL-DVT. Being more than 40 years and female had more events of LL-DVT compared to <40 years and males (P<0.05).

Introduction

Deep vein thrombosis (DVT) refers to the formation of one or more thrombi in one of body's large veins most commonly in the lower limbs. Proximal DVT refers to involvement of popliteal veins or above and isolated calf vein thrombosis is considered as distal vein thrombosis.[1] The clinical conundrum is that symptoms (pain and swelling) are often nonspecific or absent. However if left untreated a thrombus could become fragmented or dislodged and migrate to obstruct the arterial supply to the lungs causing potentially life threatening pulmonary embolism. Over the past 25 years the pathophysiology of DVT has become much better understood. Over a century ago Rudolf Virchow described three features which are critically important in the development of venous thrombosis[2]; venous stasis, activation of blood coagulation, and venous damage.

These factors are known as the "Virchow Triad". Under normal circumstances a physiologic balance is present between factors that promote and retard coagulation. A disturbance in this equilibrium may result in the coagulation process occurring at an inopportune time or location or in an excessive manner. Numerous factors, often in combination contribute to DVT. These may be categorized as acquired or inherited. Acquired risk factors could be of two types and include transient acquired risk factors (e.g. pregnancy, post-operative) and non-transient acquired risk factors (cancer, antiphospholipid syndrome). The diagnosis of DVT historically required venography which is expensive and invasive. Even though venography is considered the criterion of standard since 1990 most widely used method for diagnosis is non-invasive sonographic examination.[3] The simple and cheaper D-dimer test has been validated as an initial screening test and confers a high negative predictive value when it is negative with a low clinical probability.[1]

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Early recognition and appropriate treatment of DVT and its complications can save many lives. Similarly recognition of high risk individuals and initiation of thromboprophylaxis can be not only lifesaving but also much more cost effective. There is no country data for hospital admission rates for DVT and its pattern in Sri Lanka. The purpose of this study was to determine the hospital admission rate, pattern of lower limb DVT and its relationship to acquired risk factors among patients admitted to Colombo South Teaching Hospital during the year 2010.

Methods

A descriptive cross sectional study was carried out at the Haematology Department, Colombo South Teaching Hospital (CSTH) among patients admitted during the year 2010 with lower limb DVT (LL-DVT). All patients with LL-DVT admitted during the year 2010 were identified from the doppler ultrasound scan list available at the Radiology Department, CSTH. This was further supported by the anti-coagulation clinic register at the Haematology Department, CSTH where there is a record of all the patients referred for further management and follow up. Patients' details such as demographic data, risk factors for LL-DVT and investigations related to LL-DVT were obtained from their bed head tickets and the anticoagulation clinic book. Transient risk factors for LL-DVT such as pregnancy, postpartum period, immobility, post-operative period, intensive care unit admission, pharmacological agents, trauma, other co-morbid factors (e.g. presence of uterine fibroids) were recorded. The non-transient risk factors assessed were anti-phospholipid syndrome (APLS), cancer, varicose veins, and congestive cardiac failure, and myelo-proliferative neoplasm (MPN). Data collection was done for a period of three months. A data extraction sheet was used for data collection. All components of the data collection format were manually checked and data cleaning and coding was done. Data entry and statistical analyses were done using the software package SPSS (version 15). Ethical clearance for the study was obtained from the ethical review committee of the CSTH.

Results

Total admissions for the year 2010 to CSTH were 136,794. The total number of LL-DVT patients detected were 34. Therefore, the hospital admission rate of LL-DVT among the study population was 25 per 100,000 admissions per year. The age distribution of the study population was from 19 years to 81 years and the median age was 41 years (Table 1). Females had significantly more LL-DVT compared to males being 73.5% vs 26.5% (p<0.001). Proximal DVT was higher than distal DVT (Table 2).

Table 1. Age distribution of the study population

Age category	Number	%
< 40 yrs	14	41.2
≥ 40yrs	20	58.8
Total	34	100.0

Table 2. Distribution of the site of thrombosis

Site of LL-DVT	Number	%
Proximal	22	64.7
Distal	7	20.6
Both	5	14.7
Total	34	100.0

(LL - DVT : Lower limb deep vein thrombosis)

An acquired risk factor was present in 68% (n=23) (Figure 1). A single risk factor was present in 74% (n=17) and 26% (n=6) had multiple risk factors. Immobility was the commonest risk factor identified, followed by pregnancy and puerperium, and presence of APLS and cancer / a MPN (Table 3). When the risk factors were categorized 52 % (n=12) were transient risk factors and 48% (n=11) were non-transient (Table 4).

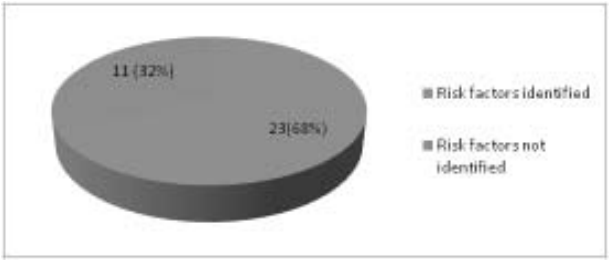

Figure 1. Distribution of risk factors in the study population

Table 3. Distribution of risk factor types

Risk factor	Number	Percentage
Pregnancy and puerperium	4	11.8
Immobility	11	47.8
Post operative	2	5.9
Intensive care unit admission	2	5.9
Antiphospholipid syndrome	4	11.8
Cancer/Myeloproliferative neoplasm	4	11.8
Varicose veins	2	5.9
Trauma	1	4.3
Congestive cardiac failure	1	4.3
Auto-immune-haemolysis	1	4.3

Table 4. Distribution of risk factors by site

Site of LL-DVT	Transient		Non Transient	
	No.	Percentage	No.	Percentage
Proximal	9	75%	8	73%
Distal	3	25%	0	-
Both	0	-	3	27%
Total	12	100%	11	100%

LL-DVT: Lower limb deep vein thrombosis

Discussion

The annual hospital admission rate of LL-DVT among the study population was 25 per 100,000 admissions. This is a rising trend compared to what White *et al.* reported in 2005 of the rate of DVT among Asians/Pacific Islanders living in California which was 21 per 100,000.[4] This may reflect the rising trend in line with studies done in Singapore which concluded that even though DVT is not common in Asians as it is in Caucasians, the prevalence now is much higher than it was earlier.[5-7] The relationship of LL-DVT to age and gender was similar to what is reported in other populations; where elderly females have a higher tendency to develop DVT.[8]

In the study population proximal LL-DVT was commoner than distal LL-DVT or LL-DVT occurring in both distal and proximal veins (Table 2). Varying results have been reported in the world literature for the frequencies of distal versus proximal vein thrombosis. Some report as distal DVT being commoner while others report an opposite view.[9,10] The diagnostic strategies looking mainly into proximal vein thrombosis could be a one reason for this observation and it is also well recognized that ultrasound scan being particularly user dependent and detection rates of distal LL-DVT vary considerably between operators and departments.[11,12] Another contributing factor for this could be variations in lower limb venous anatomy.[13]

The relationship between LL-DVT and associated risk factors were analyzed in the study. Majority 68% (n=23) had acquired risk factors. Immobility was the most common risk factor (47.8%), while malignancy/ MPN, pregnancy and APLS were also among the risk factors (Table 3). These results are similar to data reported in Asian DVT cohorts and world literature.[7,14,15] It is important to note that 26.1% of patients had multiple risk factors.

It was found that proximal LL-DVT and extensive LL-DVT (LL-DVT involving both proximal and distal veins) are commonly associated with non-transient risk factors (malignancy, APLS, congestive heart failure, autoimmune haemolysis and varicose veins). None of the patients with distal LL-DVT had non-transient risk factors (Table 4). This finding support reported data on the risk factors on distal versus proximal thrombosis.[9] Approximately equal percentages of proximal and distal LL-DVT were associated with transient risk factors; 41% and 43% respectively in this study. (9/22 patients with proximal DVT and 3/7 patients with distal DVT had transient risk factors – Table 2 and Table 4). Therefore we cannot conclude that distal LL-DVT is more commonly associated with transient risk factors compared to proximal LL- DVT.[9]

The main limitation of the study was the small sample size. Smoking is well known risk factor associated with DVT[16]. This was not analyzed as the study was mainly based on case notes and patients were not directly interviewed. An acquired risk factor was not identified in a total of 11 patients. These patients may have an acquired risk factor which was not studied or have an inherited risk factor. Inherited thrombophilia as a risk factor was not analyzed during the study.

Conclusions and recommendations

It is the standard practice in most of the developed countries including United Kingdom to assess the risk of thrombosis in hospitalized patients and provide appropriate thromboprophylaxis. It is widely accepted that this is the most cost effective way of reducing death associated with VTE, reducing the treatment cost in DVT and treatment cost in long term complications.[17] Therefore it is important for us to think about a national guideline and protocol based on the guideline for prevention of thrombosis.

Authorship

Contribution: Both authors contributed equally.

Conflict-of-interest disclosure: The authors declare no conflict of interest.

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CME Article (Series III)

Theme: Acute trauma coagulopathy and massive transfusion

HWCK Kulathilake[1], I Wijesiriwardena[1]

Questions 1 and 2 are multiple choice questions to activate your brain cells to the pathogenesis of acute trauma coagulopathy (ATC) and massive transfusion, as there are new insights into this subject.

1. Acute trauma coagulopathy
   a) is initiated by vascular endothelial damage
   b) protein C activation pathway is one of the major contributors to its pathogenesis
   c) has the same pathophysiology of disseminated intravascular coagulation (DIC)
   d) haemodilution is beneficial in prevention of ATC
   e) hypothermia aggravates the condition

2. Complications of massive transfusion include
   a) coagulopathy
   b) hypothermia
   c) hypocalcaemia
   d) hypokalaemia
   e) metabolic alkalosis

Answer Grid:

Q.1	**T T T T**	**Q.2**	**T T T T**
	F F F F		**F F F F**

CME Points: 05

The answers are given in page 33 with the explanations.

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Case report I

Paroxysmal cold haemoglobinuria in a pregnant woman leading to severe anaemia and fetal loss

EL Asfir[1], **N Senadheera**[2], **S Jayasinghe**[3], **H Liyanage**[3]

Key words: paroxysmal cold haemoglobinuria; steroids; fetal loss

Abstract

Paroxysmal cold haemoglobinuria (PCH) is an autoimmune haemolytic anaemia which gives rise to intravascular haemolysis and a varying degree of anaemia. Its presentation in pregnancy is rare but can be potentially fatal to both mother and fetus. This case report describes a pregnant woman with PCH presenting with acute severe anaemia which led to fetal loss. The mother was successfully managed with blood transfusions and steroid therapy. We stress the importance of timely intervention when managing similar patients.

Introduction

Paroxysmal cold haemoglobinuria (PCH) is a rare form of autoimmune haemolytic anaemia more commonly seen in children. Historically this was described in association with syphilis but nowadays it's mostly seen following viral or bacterial infections[1-5]. The haemolysisin PCH is mediated by a biphasic IgG autoantibody that triggers complement – mediated intravascular haemolysis.[1-4] In adults, it is rare and represents less than 1% of all autoimmune haemolytic anaemias.[6]

We report a case of a pregnant woman who presented with acute severe haemolysis and fetal loss, and diagnosed as having PCH and successfully managed with blood transfusions and steroids. To our knowledge PCH presenting in pregnancy has been reported only once before in the literature.[7]

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Case report

A 23-year-old female in her second pregnancy, presented to a local hospital at 32 weeks of gestation, with evidence of intravascular haemolysis and was transferred to our hospital, a tertiary care women's hospital for specialized care.

This presentation was while convalescing following a chickenpox infection, and she had developed fever and lower abdominal pain immediately after a cold bath. This was associated with passage of dark urine. On admission to the local hospital, she was very pale and icteric and the haemoglobin (Hb) level was 54 g/L. As facilities for transfusion was not available in this hospital she had to be transferred to the nearest provincial general hospital. There the investigations carried out detected a positive direct antiglobulin test (DAT) and indirect hyperbilirubinaemia. By this time she complained of reduced fetal movements and an ultrasound scan done showed absence of fetal heart beat confirming an intrauterine fetal death (IUD). As compatible blood for transfusion could not be provided she was transferred to our hospital.

On arrival she was drowsy, dyspnoeic, pale, icteric, blood pressure was 85/50mmHg. The Hb level was 24 g/L, packed cell volume (PCV) 6.1%, white cell count (WBC) 17.3×10^9/L, with neutrophils – 78% and lymphocytes – 21%. Platelet count – 437×10^9/L, indirect bilirubin level – 8.4mg/dL and the potassium level – 6.8mmol/L. Blood picture showed red cell agglutination, many spherocytes, occasional polychromatic cells, occasional fragmented red cells and neutrophil erythrophagocytosis. (Figure 1 & 2). Absolute reticulocyte count was 0.6×10^9/L and reticulocyte index was 0.2%. Serum Lactate Dehydrogenase (LDH) level was elevated (3250u/L). The DAT was strongly positive with anti-C3d but negative with anti-IgG. Mycoplasma antibody was negative. Indirect Donath-Landsteiner (DL) test was positive confirming PCH.

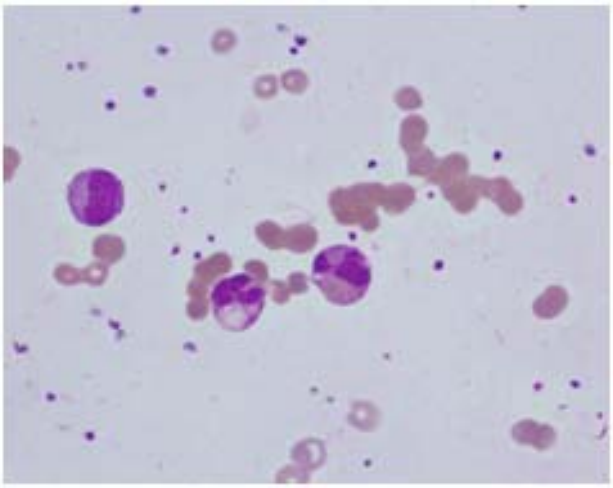

Figure 1. Peripheral blood smear (Leishman stain, x 100) – Showing erythrocyte autoagglutination.

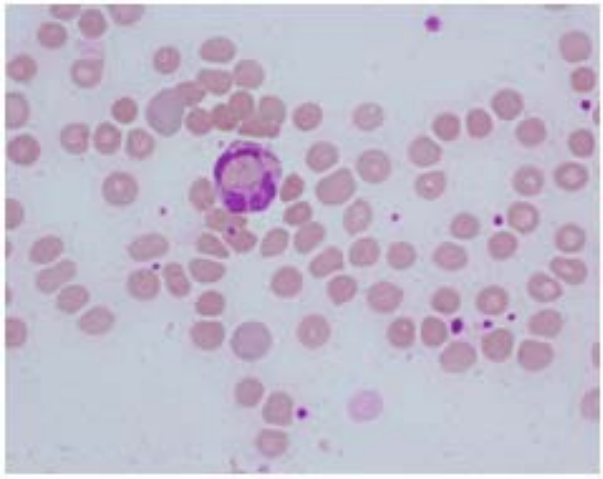

Figure 2. Peripheral blood smear (Leishman stain, x 100) – Showing erythrophagocytosis by neutrophils.

She was managed in the Intensive care unit as she was drowsy and dyspnoeic, and then was kept warm and two units of uncross matched O negative packed red blood cells were given soon after admission via the blood warmer and followed by one unit of cross matched washed O positive red cell concentrates. Folic acid 5 mg daily was commenced on the same day. At the end of day one the Hb level increased to 80g/L. The dead fetus was delivered spontaneously on the following day.

On the second day the Hb level dropped again to 69 g/L, and there was a persistently low absolute reticulocyte count of 1.7 x 10^9/L and reticulocyte index was 0.7%. This day the urine output reduced to < 0.5ml/kg/h. Another unit of blood was transfused. Despite the blood transfusion the Hb level continued to fall and dropped from 80 g/L to 71 g/L the next 24 hours. Therefore intravenous (IV) methyl prednisolone (MP) 500mg daily was commenced on day three. Following commencement of IVMP, the Hb level improved over the next four days to 90 g/L without any further blood transfusions and the LDH level gradually came down to 1400U/L (Figure 3). The IVMP was given for 3 days followed by oral prednisolone 40mg daily. On discharge she was referred to the local hospital for follow up. Prednisolone was tailed off over a few weeks. Her Hb level remained stable at her follow up visit to the local hospital.

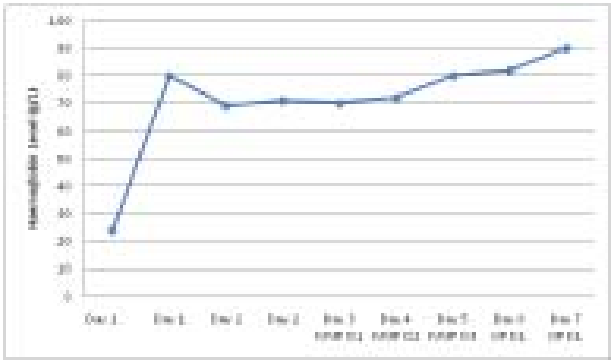

Figure 3. Showing the temporal profile of the haemoglobin level.

(IVMP – Intravenous methyl prednisolone, OP – Oral prednisolone, LDH – Lactate dehydrogenase level)

Discussion

At the time PCH was first described by Donath and Landsteiner in 1904, it was a chronic relapsing condition affecting mainly adults and 90% were secondary to syphilis. Since then the presentation has changed due to the effective treatment of syphilis and now PCH is seen mostly in children as a post viral syndrome commonly following infections such as chickenpox, mumps and measles. Less commonly, bacterial infections with *Haemophilus influenzae, Escherichia coli* and *Staphylococcus aureus* have been implicated[3,8]. The acute non-relapsing form of PCH has been documented to occur in adults[9].

Our patient had typical laboratory findings of PCH. Her DAT was positive for complement C3d but negative for IgG. Although DL antibodies are of IgG class, this is a common finding in PCH. There are also reported cases where DAT has been negative. Non detection of DL antibodies can be due to low-affinity of the IgG, the immunoglobulins being RBC-bound, or IgG levels being below the threshold level of the test. Another postulation is that the IgG autoantibodies are directed against reticulocytes or mature RBC precursors rather than red blood cells, which is based upon the presence of reticulocytopenia in some cases.[10] Use of special blood bank techniques may overcome some of these false negativities.

Our patient had a relative reticulocytopenia which again is a known finding in PCH.[11] This reflects an ineffective erythropoiesis either due to destruction of precursor red cells by DL antibodies or a result of viral haematopoietic suppression.

A striking feature in the peripheral blood film was the erythrophagocytosis by neutrophils. Although erythrophagocytosis by monocytes occur in autoimmune haemolytic anaemia of various aetiologies, erythrophagocytosis by neutrophils is most strongly associated with PCH.[12] Other cases where this has been reported in are cold haemagglutinin disease, incompatible blood transfusions, haemolytic disease of the newborn and potassium chloride poisoning.[12]

Paroxysmal cold haemoglobinuria is usually self limiting and requires only supportive care during the period of acute haemolysis. However the degree of haemolysis may vary between patients because the characteristics of the haemolytic antibody are highly variable. The amount of the antibody and the affinity of the antibody for the red blood cell surface are important determinants of the severity of haemolysis. Furthermore, some antibodies may fix complement less efficiently.[6] IgG antibodies can cross the placenta and result in haemolysis in the fetus. Our patient experienced severe haemolysis and anaemia leading to IUD of the fetus.

Management includes avoidance of cold temperature, folic acid supplements and transfusion of red cell concentrates. Uncross matched group O blood can be given until group compatible blood is available after cross matching at 37°C. Utilizing washed RBC units has not been proven to improve transfusion safety, but this can be performed if patient's condition remains refractory to standard warmed blood transfusions.[13]

The severity of anaemia and the reticulocytopenia prompted the initiation of steroids in our patient resulting in a dramatic response. There is no uniform consensus on the role of steroids in the management of PCH but several reports show that it has been used successfully in similar circumstances.[10] Usually, a remission can be seen in 1 to 3 weeks. Once the haemolysis is controlled, corticosteroids can be tapered. Close monitoring for relapses is required for a few weeks, with slowing of corticosteroid taper if signs of possible relapse develop.

The other available treatment options are plasmapheresis and rituximab therapy.[10,14] Given the transient and relatively brief production of DL antibodies after a viral infection, clearance of the antibody may be possible with plasma exchange. Another reason for the effectiveness of plasmapheresis in this condition is that the antibody preferentially binds to the RBC, shifting the antibody equilibrium to the intravascular component, allowing easy removal.[10] Rituximab has also been used successfully to treat relapses of chronic PCH in an adult patient, where disease was unresponsive to steroids.[14] Splenectomy is usually ineffective as the haemolysis is intravascular.

An important issue which was evident in the presentation of this patient was a lapse in the emergency transfusion facilities in peripheral hospitals. The DAT positivity can pose problems for the blood bank when selecting red cell units by routine methods. However it needs to be emphasized that transfusion of uncrossmatched group O blood in such situations is justified and can be lifesaving[15].

Authorship

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Case report II

Lupus anticoagulant – hypoprothrombinaemia syndrome

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Key words: bleeding, lupus anticoagulant, acquired hypoprothrombinaemia, systemic lupus erythematosus

Abstract

Lupus anticoagulant – hypoprothrombinaemia syndrome (LA-HPS) is a rare acquired disorder caused by the combination of lupus anticoagulants and anti-prothrombin antibodies. A 6-year-old girl was admitted with intractable bleeding from a tooth. Both prothrombin time (PT) and activated partial thromboplastin time (aPTT) were prolonged with test to control ratio > 3 and aPTT did not get corrected following 50:50 mix with pooled normal fresh plasma (PNP); the inhibition was not time dependent. The PT got corrected following 50:50 mix. Both dilute Russell viper venom time (dRVVT) and kaolin clotting time (KCT) were strongly positive. Antinuclear antibody (ANA) and anti-double stranded DNA (anti-dsDNA) were positive. Based on the coagulation derangements and positivity for the lupus anticoagulant (LA) and ANA and DS/DNA the diagnosis of the rare lupus anticoagulant-acquired hypoprothrombinemia syndrome (LA-HPS) was made. The patient was treated with high dose corticosteroids and normal coagulation test results were obtained.

Introduction

Lupus anticoagulant (LA) is a group of antibodies directed against phospholipid binding proteins. These phospholipids are essential for the activation of main steps of the coagulation cascade.[1] The LA, results in thrombosis in-vivo than bleeding due to activation of phospholipids and binding proteins, endothelial activation and inactivation of natural anticoagulants.[1] The LA may manifest with bleeding when associated with an acquired factor VIII inhibitor or rarely due to thrombocytopenia, or an acquired thrombocytopathy.[2-4] Lupus anticoagulant – hypoprothrombinaemia syndrome (LA-HPS) is a rare acquired disorder caused by prothrombin antibodies, in adults or in children mostly associated with systemic lupus erythematosus (SLE), has been reported to manifest with a bleeding diathesis caused by factor II(FII) deficiency.[3,4]

We report the clinical case of a girl whose onset of SLE was a bleeding manifestation caused by a serious coagulopathy with prolonged PT and aPTT in the presence of LA due to a LA-HPS.

Case report

A 6-year-old girl was admitted to ward 09, of the Lady Ridgeway Hospital with history of prolonged and excessive bleeding following an accidental injury to a tooth. The clinical examination was unremarkable other than the mild bleeding from the tooth socket. The girl had no past history of congenital coagulopathy or other important illnesses. She had no family history of any bleeding disorder. She did not give a history of or have clinical features to indicate SLE.

The laboratory investigations on admission were as follows: Full blood count haemoglobin (Hb) level 131 g/L, red blood cells (RBC) 4.98 x 10^{12}/L, mean cell volume (MCV) 77.8fL, mean cell haemoglobin (MCH) 26.2 pg, mean cell haemoglobin concentration (MCHC) 33.7 g/dl, white blood cell count (WBC) 12.6 x 10^9/L with neutrophils -56%, lymphocytes -34% and monocytes 4%, and platelet count 410 x 10^9/L. Erythrocyte sedimentation rate (ESR) 74 mm 1st hr. Blood picture: RBC showed moderate rouleaux formation and appeared normochromic normocytic. WBC and platelet morphology were unremarkable. Reticulocyte count was 1%. Serum bilirubin levels were normal. Coagulation tests: Bleeding time – 3 minutes (Control 0-6min), PT 27.5s (control 11.0s), aPTT 81.7s (control 26.0s), thrombin time (TT) 18.2s (control 15.6s). Fibrinogen level (antigen) 3.3 g/L (normal range 1.5- 4.0/L) and

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fibrinogen activity by Clauss method 372 g/L (normal range 150- 400g/L). Correction studies with pooled normal fresh plasma (PNP) (50:50 mix): PT was corrected, and the aPTT remained prolonged without correction. The inhibitor screening: negative (difference of aPTT following 50:50 mix with PNP at and after 2 hours incubation was < 6 sec). Lupus anticoagulant (LA) screening: dRVVT- LA ratio 2.3 (normal < 1.2) and KCT test >5 min, control 82s. Liver function tests and renal function tests were normal. Urine analysis normal with no red cell casts. Direct antiglobulin test (DAT) was positive with C3d specificity. Anti-nuclear antibody (ANA) was positive with a titre > 1/80 by direct immunofluorescence method. The ds-DNA antibody was positive by enzyme linked immunosorbent assay method.

Prolongation of aPTT without being corrected following PNP mix indicated presence of a coagulation inhibitor. The presence of a time dependent inhibitor was excluded by the 2 hour incubation test. Both strongly positive dRVVT and KCT tests indicated the presence of LA. Positive ANA, ds-DNA and DAT indicated the presence of an autoimmune disease. Although the clinical criteria were not fulfilled for SLE and factor II assay and prothrombin antibody level was not performed due to the unavailability of the facilities to test; based on the test results diagnosis of the rare lupus anticoagulant – acquired hypoprothrombinemia syndrome (LA-HPS) was made. Repeating of the ANA, ds-DNA and the dRVVT test was planned to confirm persistent positivity after 12 weeks.

The patient was treated with oral prednisolone 1mg/kg/day and the coagulation test results reverted to normal within 2 weeks of treatment (Table 1).

Table 1. Coagulation test results from the date of admission

Days Vs Tests	**Day 1**	**Day 5**	**Day 10** **Day 1 of treatment**	**Day 24** **Day 14 of treatment**
PT test PT Control	22s 13s	27s 11s	37.1s 13.8s	14.3s 11.0s
aPTT test aPTT Control	71s 27s	81s 26s	144s 34.8s	37s 30s
TT test TT Control	-	18s 15.6s	-	-
PT following 50:50 mix with PNP	-	17s (corrected)	21s (corrected)	11.1s
aPTT following 50:50 mix with PNP	72s Not corrected	67.6s Not corrected	126s Not corrected	32s corrected
Inhibitor screening (aPTT 50:50 mix with PNP) Fresh Mix (At) Incubated mix (Affter 2hrs)	-	Negative 76.2s 78.2s		
dRVVT LA1(Screening test) LA2 (Confirmation test) LA1/LA2 Ratio	-	 262s (Cont:40s) 110s (Con:36s) 2.3 (1.2) (Strongly positive)	- -	-
KCT KCT 50:50*		>5 min >5 min	 -	 -

*50:50 correction = mixture of patient's plasma and pooled normal fresh plasma (PNP)at a ratio of 1:1, PT - prothrombin time, aPTT - activated partial thromboplastin time test, TT - thrombin time, dRVVT = dilute Russell viper venom time, KCT = kaolin clotting time, LA 1 = lupus anticoagulant screening reagent 1, LA 2 = lupus anticoagulant screening reagent 2, S = seconds, Min = minutes

Patient was followed up at the paediatric clinic and the haematology clinic regularly with the assessment of clinical parameters and the relevant haematological investigations.

Discussion

Lupus anticoagulant is the coagulation based demonstration of antiphospholipid antibodies by phospholipid dependent coagulation tests dRVVT and KCT. Hypoprothrombinaemia results in prolongation of both PT and aPTT with normal TT and corrected by mixture of patient's plasma and normal plasma at a ratio of 50:50 which can be confirmed by the factor II level assay.[3-6]

The LA-HPS is a rare acquired disorder associated with haemorrhagic manifestations. It appears mostly in young females, even in infants. It presents as a complication of SLE or transient viral infections.[2,3]

The LA-HPS is characterised by a very strong LA and presence of polyspecific antibodies. They bind to the epitopes of the anionic phospholipids and of prothrombin, but they do not neutralise prothrombin. Therefore, a FII activity deficiency is not due to an inhibitor, as suspected, but to an evident factor decrease owing to the higher clearance of the prothrombin-antibody complex in the reticuloendothelial system.[3,4]

Corticosteroid therapy is the treatment of choice for LA-HPS associated with SLE; it normalizes the PT time.[2-4] In cases associated with a viral infection, LA-HPS reverses spontaneously with the resolution of the infection. Therefore, steroid therapy is not necessary.[2-4] Given the complex coagulopathy of our patient, caused by an acquired inhibitor, and the risk of serious major haemorrhage, we commenced steroid therapy and was able to obtain complete normalisation of PT and APTT after of two weeks of treatment.

Finally, we conclude that, LA not only produces APTT prolongation, but may also produce a more complex coagulopathy with PT prolongation-LA-HPS type. LA-HPS onset could be haemorrhage related with an added FII deficiency and marked inhibitor effects of APTT dependent factors. In our patient LA-HPS was the first and the most important clinical sign of the onset of the systemic illness. Our patient's LA-HPS was successfully treated with steroids, confirming that this treatment represents the leading therapy for this coagulopathy.

Authorship

Contribution: All authors contributed equally.

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Images in Haematology

A case of Large Granular Lymphocytosis

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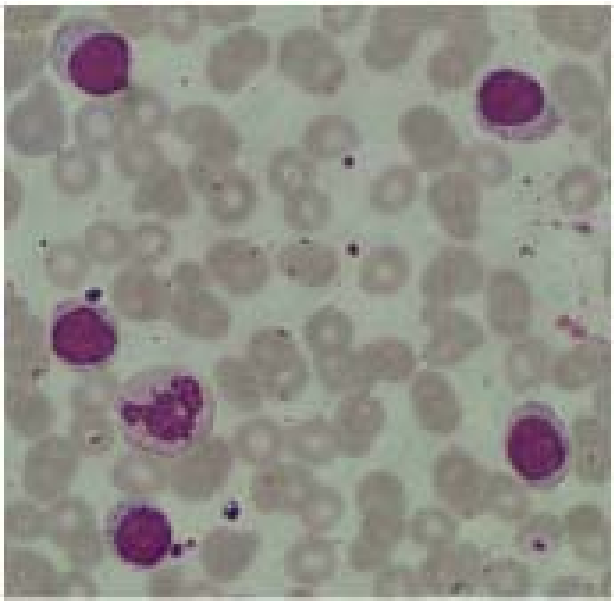

Figure 1. Peripheral blood smear (Leishman stain, ×100) – a monomorphic population of moderate to large lymphocytes with abundant pale cytoplasm containing many azurophilic granules, eccentric nuclei with condensed chromatin.

A 65-year-old previously well female without lymphadenopathy or organomegaly had peripheral blood lymphocytosis of $11.6x10^9$/L. Total white cell, neutrophil and platelet counts were $14.9x10^9$/L, $2.9x10^9$/L,$250x10^6$/L respectively. Haemoglobin level was 11.4g/dL. Lymphocyte morphology resembled large granular lymphocytes (LGL) (Figure 1). Marrow aspirate showed 70% cells with LGL morphology, and immunophenotype was CD3+(89%), CD8+(84%), CD4+ (80%), CD57+(80%), CD2+(94%), CD5+(88%), CD56-, CD7-TCRβ+(89%) and TCRγδ-. Marrow biopsy had a 30% interstitial infiltrate of lymphocytes being CD 3+, CD5 +, CD 57+ and scattered positive for CD20/CD8.

Cellular morphology favored T or Natural Killer (NK) cell disorder. Immunophenotype was compatible with T cell large granular lymphocytic leukemia (T-LGL).

LGL proliferations are either nonclonal in aetiology (viral infections, auto immune conditions, post splenectomy, bone marrow or solid organ transplantation) or clonal (aggressive T-LGL leukaemia and chronic or aggressive NK cell leukaemia). Clinical presentation and immunophenotype help to determine the diagnosis.

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Answers for the CME: Acute trauma coagulopathy and massive transfusion

1. TTFFT

Coagulopathy of trauma is a distinct entity with a strong impact on the outcome. Physiological haemostasis controls blood fluidity and rapidly induces haemostatic plug formation in order to stop bleeding. Primary haemostasis (vascular and platelet mechanism), coagulation and fibrinolysis are closely linked to each other and precisely regulated in order to efficiently close vessel wounds, promote vascular healing and maintain vessel patency.

There is limited understanding of the mechanisms by which tissue trauma, shock and inflammation initiate trauma coagulopathy. Acute coagulopathy of trauma-shock should be considered as a distinct entity from disseminated intravascular coagulation (DIC) as described in other conditions. Rapid diagnosis and directed interventions are important in prevention.

Coagulopathy of trauma is a haemostatic and haemodynamic depletion which is now recognized as acute trauma coagulopathy (ATC) and iatrogenic coagulopathy (IC).

Acute trauma coagulopathy has recently been recognized as a multifactorial primary condition. The principle drivers of ATC are tissue trauma, inflammation, hypo-perfusion/shock and acute activation of the neurohumoral system. The principle mechanisms of early ATC are (i) activation of the protein C pathway (ii) endothelial injury (iii) hyperfibrinolysis (iv) platelet dysfunction. Activation of the protein C pathway and endothelial damage initiates the coagulation system which ultimately leads to primary hyperfibrinolysis in ATC.

Endothelial injury may also be linked to 'autoheparinisation' as the entire endothelial glycocalyx contains approximately 1 liter of non circulating plasma with significant amount of 'heparin like substances'. When degranulated this automatically leads to autoheparinisation. Therefore, initially the pathophysiology is not like in DIC but DIC can occur secondarily in ATC. When ATC becomes exacerbated by hypothermia, acidosis and haemodilution (due to the rapid infusion of colloids) it is called IC. This then becomes a vicious cycle, enhancing more protein C activation and damage to endothelium.

It has been shown that early recognition of acute trauma coagulopathy, accompanied by appropriate and aggressive management, can correct coagulopathy, control bleeding, reduce blood product use and improve outcome in severely injured patients.

2. TTTT

Massive transfusion is associated with numerous and significant complications. In massive transfusion, coagulopathy is frequently seen due to dilution of clotting factors and dilutional thrombocytopenia. This is exacerbated by the consumptive coagulopathy triggered by massive haemorrhage in trauma.

Multiple factors contribute to hypothermia in trauma patients. Rapid transfusion of packed red cells stored at 4°C will lower the recipient's core temperature. Hypothermia leads to decreased production of clotting factors, platelet dysfunction, impaired haemostasis and decreased citrate metabolism (citrate toxicity).

Hypocalcaemia occurs due to citrate in additive solution in packed red blood cells (PRBC), fresh frozen plasma and platelets, especially in the presence of hypothermia.

Potassium concentration increases in stored PRBC, due to membrane Na^+-K^+ ATPase pump inactivation. Therefore, transient hyperkalemia is common with rapid transfusions. Hypokalemia has been noted with massive transfusion due to restoration of membrane ATPase pump thus allowing potassium to re-enter the red cells.

Citrate undergoes hepatic metabolism to bicarbonate and during a massive transfusion a metabolic alkalosis may occur.

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