Current applications of therapeutic phlebotomy

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Introduction

Phlebotomy, known also as bloodletting or venesection, is a major therapeutic procedure that has been performed by physicians in various civilisations since antiquity up to the present[1,2]. In the past it was practised using cupping, lancets or by the application of leeches[2] . This procedure often weakened the patient and resulted in his or her death. A famous example is that of President George Washington who died in 1799 following the removal of approximately 1.7 litres of blood during a bloodletting procedure for acute epiglottitis[3]. Originally, several thousand years ago, phlebotomy was used for the treatment of various disorders, but in addition to its therapeutic benefits, phlebotomy also had a preventive role. In present day medicine, phlebotomy can be performed in physicians' offices, at a blood bank or in hospital under the supervision of a doctor after obtaining a medical prescription stating the indication and number of phlebotomy sessions required. Currently, therapeutic phlebotomy is approved for three main indications: haemochromatosis, polycythaemia vera and porphyria cutanea tarda. It has also been used as a treatment alternative for many other diseases in various countries, especially in Chinese medicine, although these indications are not approved by western medicine.

We searched PubMed and Medline using the terms "phlebotomy", "bloodletting" and "venesection", limiting our search to all article types that included the indications for therapeutic phlebotomy. We came across only one review published in a nursing journal that mentioned the three main indications of therapeutic phlebotomy[4]. This review focuses not only on the three main indications but also discusses some of the other possible indications.

Polycythaemia

Polycythaemia or erythrocytosis is a term used to describe an increase in the red blood cell mass. It can be divided into absolute polycythaemia defined as an increase in the number of red blood cells, or relative polycythaemia caused by a decrease in the plasma volume. Polycythaemia can be suspected in any person with an increase in haematocrit (more than 52% or 48% in men and women, respectively), an increase in haemoglobin level (more than 18.5 g/dL or 16.5 g/dL in men and women, respectively) or an increase in red blood cell count[5].

Relative polycythaemia is characterised by a decrease in plasma volume which causes an apparent increase in the red blood cell mass. Any condition causing fluid loss, such as any cause of dehydration and severe burns, will result in relative polycythaemia. Therapeutic phlebotomy is not indicated for these patients. Apparent polycythaemia, also known as Gaisbock's syndrome, belongs to the group of relative polycythaemias; this syndrome usually affects sedentary, obese men and it has been associated with hypertension, smoking, excessive alcohol intake and use of diuretics[6]. These patients may have an increased risk of thrombotic complications. For some time now, bloodletting has been considered an essential part of the treatment of patients with haematocrit values greater than 54%, especially in patients who also have thrombotic risk factors[7]. Humphrey and his colleagues demonstrated that small volume phlebotomy (250 mL) can be safely done once every 2 months to lower the haematocrit[8].

Absolute polycythaemia can be divided into primary polycythaemias, which include polycythaemia vera (PV), and secondary polycythaemias.

PV belongs to the group of myeloproliferative disorders; it is characterised by the presence of an increased red cell mass or haematocrit and sometimes increases in the white blood cell and platelet counts. It tends to affect the elderly although it can occur in any age group. PV is distinguished from secondary polycythaemias by the presence of a low serum erythropoietin level[9]. PV is suspected in any patient with an elevated haemoglobin level (more than 16 g/dL in women or 18 g/dL in men) or haematocrit (more than 47% in women or 52% in men), splenomegaly with or without leucocytosis and thrombocytosis, and in patients presenting with portal venous thrombosis[9]. However patients can be asymptomatic. Other signs and symptoms include pruritus after a warm bath, weakness, weight loss, gouty arthritis and peptic ulcer disease. Erythromelalgia, which is an acute onset of a burning sensation that affects the four limbs and is usually accompanied by a reddish or bluish colouration, is a rare but classic symptom of both PV and essential thrombocythaemia[10]. The diagnosis of PV can be established using the criteria of the Polycythaemia Vera Study Group (PSVG)[5] or the World Health Organisation (WHO)[11] after excluding all causes of secondary polycythaemia (Tables I and II).

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Table I - Diagnostic criteria for polycythaemia vera according to the Polycythaemia Vera Study Group (PVSG) and World Health Organisation (WHO) criteria.

Polycythaemia Vera Study Group[5]	WHO criteria[11]
Major criteria	**Major criteria**
- Increased red cell mass Males: ≥36 mL/kg Females: ≥32 mL/kg - Arterial oxygen saturation ≥92 per cent - Splenomegaly	- Haemoglobin >18.5 g/dL in men, >16.5 g/dL in women or other evidence of increased red cell volume - Presence of $JAK2^{V617F}$ or other functionally similar mutation such as JAK2 exon 12 mutation
Minor criteria	**Minor criteria**
- Platelet count $>40\times10^9$/L - White blood cell count $>12\times10^9$/L - Leucocyte alkaline phosphatase score >100 - Serum vitamin B12 >900 pg/mL or serum unbound B12 binding capacity >2,200 pg/mL	- Bone marrow biopsy showing hypercellularity for age with trilineage myeloproliferation - Serum erythropoietin level below the normal reference range - Endogenous erythroid colony formation in vitro
Diagnosis requires the presence of all three major criteria or the first two major criteria and any two minor criteria	Diagnosis requires the presence of both major criteria and one minor criterion or the first major criterion and two minor criteria

Table II - Causes of secondary polycythaemia.

Hypoxia
Cyanotic congenital heart disease (Eisenmenger's syndrome, tetralogy of Fallot)
Chronic obstructive pulmonary disease
Obstructive sleep apnoea
Living at high altitudes
Carbon monoxide poisoning
Cigarette smoking
Renal disease
Post-renal transplant
Renal artery stenosis
Polycystic kidney disease
Tumours
Hepatocellular carcinoma
Renal cell carcinoma
Von Hippel Lindau syndrome
Phaeochromocytoma
Cerebellar haemangioblastoma
Iatrogenic
Erythropoietin administration
Anabolic steroids
Testosterone replacement therapy

Patients with PV tend to develop thrombotic events such as cardiovascular and cerebrovascular accidents, and arterial and venous thromboembolism; moreover, the course of the disease can be complicated by myelofibrosis and/or evolution into acute myeloid leukaemia/myelodysplastic syndrome[12]. One of the major goals of treatment is to reduce these thrombotic events; the median survival for treated patients is currently over 10 years. Several trials have investigated the outcomes of various therapeutic combinations and they all concluded on the importance of therapeutic phlebotomy. The most important one was the PVSG prospective trial in which 400 patients were randomly assigned to receive either phlebotomy alone or chlorambucil with phlebotomy as needed or radioactive phosphate (^{32}P) with phlebotomy as needed and were then followed for 20 years. The median survival was 13, 11 and 9 years for patients randomly assigned to treatment with phlebotomy alone, radioactive phosphate and chlorambucil, respectively[13,14]. The study also showed an increased incidence of thrombosis among the group treated with phlebotomy alone, especially during the first 3 years (23% compared to 16% in the ^{32}P treatment arm). However, compared to patients given myelosuppressive therapy, patients who were treated with phlebotomy alone had a lower incidence of haematological malignancies and solid tumours. The authors concluded that phlebotomy provides the best overall survival but at an expense of increased risk of thrombosis during the first 3 years[13,14]. To resolve the issue of thrombosis, another trial was conducted in which patients were given high-dose aspirin and dipyridamole in addition to phlebotomy, but it was found that this addition of high doses of anticoagulants increased the incidence of gastrointestinal haemorrhages[15]. However, low-dose aspirin (81 mg) decreased the risk of various thrombotic events. Hydroxyurea can be used for maintenance therapy in patients who are at high risk of thrombosis or in those who cannot tolerate therapeutic phlebotomy[16]. Other therapeutic options include treatment with interferon-alpha, or with anagrelide, which is used for essential thrombocythaemia.

Phlebotomy is now considered to be the mainstay of PV treatment. Side effects that may occur following therapeutic phlebotomy are identical to those after any blood donation. The difference is that phlebotomy is done more frequently than voluntary blood donation and therefore patients often report being fatigued and dizzy after several sessions. Iron deficiency may

develop but it is usually a mild self-limiting anaemia and iron supplementation is not required unless it become symptomatic. In one study that involved 1,000 blood donors who were interviewed 3 weeks after whole blood donations, the most common reported adverse events were arm bruises, followed by arm soreness, fatigue, vasovagal reactions, haematoma, nausea and vomiting[17]. Therapeutic phlebotomy has some limitations: patients may be intolerant, or have a low acceptance of it and it may be difficult to gain peripheral vein access. There are no absolute contraindications; the relative contraindications include severe heart disease and anaemia. At our institution, during each session, 450 mL of blood are withdrawn daily until the haematocrit drops below 40%; this is usually followed by maintenance phlebotomy at regular intervals every 1 to 2 months according to the haematocrit level. However, the interval between phlebotomies varies widely and may be much longer than every 2 months.

There are no true guidelines concerning the optimal haematocrit level in patients with PV. Some studies suggested maintaining haematocrit at a level below 45% to reduce the risk of vascular occlusive episodes[18]. Thomas and his colleagues showed that a reduction of the haematocrit to a mean of 45.5% was associated with a decrease in whole blood viscosity with a great improvement of cerebral blood flow (73%; P<0.001)[19]. However a recent study conducted by Di Nisio *et al.* found no correlation between haematocrit levels and thrombotic episodes or mortality in patients with PV[20]. In order to determine the optimal cut-off for haematocrit level, a large trial (CYTO-PV trial) was conducted in Italy[21]. This trial showed that patients maintained at a target haematocrit of less than 45% had a significantly lower rate of cardiovascular death and major thrombosis compared to those maintained at a haematocrit greater than 45%, which contrasts with the findings of Di Nisio *et al*. In this large trial, 182 adults with JAK2-positive PV were randomly assigned to the low-haematocrit group (haematocrit <45%) and 183 adults with JAK2-positive PV to the high-haematocrit group (haematocrit >45%). Some patients received phlebotomy every other day or twice a week until the target haematocrit was reached, some were administered hydroxyurea and some were treated with both therapies. Cardiovascular events occurred in 4.4% of patients in the low-haematocrit group and 10.9% of those in the high haematocrit group (hazard ratio, 2.69; 95% CI: 1.19 to 6.12; P=0.02) while the incidence of death from cardiovascular causes or major thrombosis was 1.1 per 100 person-years in the low-haematocrit group and 4.4 per 100 person-years in the high-haematocrit group. This trial showed that a haematocrit less than 45% is associated with a lower rate of thrombotic events[22].

Secondary polycythaemias

Secondary polycythaemias are characterized by an increase in the erythropoietin level, which distinguished them from PV. Many conditions may result in secondary polycythaemia but not all patients are candidates for phlebotomy. Altitude, smoking, renal cell carcinoma, hepatocellular carcinoma, adrenal adenoma, von Hippel-Lindau disease, Cushing's syndrome and phaeochromocytoma can cause secondary polycythaemia but these are not indications for phlebotomy (Table II). Similarly, patients with hypogonadism on testosterone therapy and athletes on anabolic steroids who develop secondary polycythaemia are not candidates for phlebotomy. However, phlebotomy can be performed in hypoxic conditions such as chronic lung diseases and cyanotic heart disease. Historically, phlebotomy has been used for the treatment of secondary polycythaemia in patients with chronic obstructive pulmonary disease. It results in an improvement of cerebral perfusion as well as sensory and mental function by lowering blood viscosity[23]. Moreover, it can improve oxygen consumption significantly (P<0.05) without affecting oxygen delivery[24]. It results in an increase in cardiac output with improvement of exercise tolerance as well as a reduction in the severity of angina pectoris[25,26]. A recent case report of one patient demonstrated more rapid weaning from a ventilator, with earlier extubation, in an intubated patient with chronic obstructive pulmonary disease[27]. Patients with hypoxic pulmonary disease who have hyperviscosity symptoms or a haematocrit greater than 56% should have phlebotomy to reduce this value to 50-52% (grade B recommendation: evidence level III). However, long-term oxygen therapy may be better (grade A recommendation: evidence level 1A)[28].

Renal transplant patients may develop hypertension with erythrocytosis and phlebotomy can be used is such patients when they do not respond to angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers, or can be used in conjunction with these medications in the presence of a high haemoglobin concentration. Phlebotomy was also found to reduce systolic and diastolic blood pressures significantly in these patients (P<0.01)[29]. The pathogenesis of post-transplant erythrocytosis is not fully understood but it appears to be multifactorial. At least three hormonal systems play a role: erythropoietin, endogenous androgens, and the renin-angiotensin systems. First, it was found that erythropoietin was elevated in patients following renal transplants and that erythroid progenitors had increased sensitivity to erythropoietin *in vitro*. Secondly, in vivo administration of renin or angiotensin II caused an increase in erythropoietin secretion. Lastly, endogenous androgens directly stimulate erythroid progenitors and can promote

erythropoiesis indirectly by activating the renin-angiotensin system[30]. Treatment for post-transplant erythrocytosis includes angiotensin-converting enzyme inhibitor or an angiotensin II receptor antagonist, or vensection with a target haematocrit less than 45% (grade C recommendation: evidence level IV)[28].

Patients with a cyanotic congenital heart disease, such as Eisenmenger's syndrome or tetralogy of Fallot, develop erythrocytosis secondary to their cyanosis. The haematocrit may reach levels greater than 65% and symptoms of hyperviscosity may develop. In 2008, the American College of Cardiology/American Heart Association published guidelines and recommended performing therapeutic phlebotomy for symptomatic patients with haemoglobin and haematocrit values greater than 20 g/dL and 65%, respectively, while taking care to avoid iron depletion[31]. Patients with cyanotic heart disease benefit from fluid replacement during phlebotomy and isovolaemic venesection is also recommended when a patient has symptoms of hyperviscosity but no general target haematocrit level can be suggested (grade B recommendation: evidence level III). Care should be taken because excessive phlebotomy may produce iron deficiency, which may in turn compromise oxygen delivery and raise the viscosity for a given level of haemoglobin and thus cause recurrence of the symptoms. In addition, iron replacement therapy should be used cautiously as it may provoke a rapid rise in the haematocrit level (grade B recommendation: evidence level III)[28].

Haemochromatosis

One of the main indications for therapeutic phlebotomy is haemochromatosis, but it should be noted that haemochromatosis can be divided into primary or secondary forms. Hereditary haemochromatosis, often referred to as HFE haemochromatosis, or type 1, accounts for the majority of the primary group, while the others are referred to as non-HFE haemochromatosis[32]. Others types of primary haemochromatosis include juvenile haemochromatosis, also known as type 2 haemochromatosis, which appears at a younger age compared to type 1. Juvenile haemochromatosis can itself be subdivided into two subgroups, type 2A and type 2B, according to the mutated gene, haemojuvelin (*HJV*) and hepcidin antimicrobial peptide (*HAMP*) or *HFE2B*, respectively[32]. Type 3 haemochromatosis is due to mutations that inactivate the transferrin receptor-2 (TFR2 or HFE3)[32,33]. Type 4 haemochromatosis and African iron overload are caused by mutations in the ferroportin gene known also as solute carrier family 40 (iron regulated transporter), *SLC40A1* (formerly known as *SLC11A3*[34,35]. Other types include neonatal haemochromatosis (also called gestational alloimmune liver disease, GALD), acaeruloplasminaemia, congenital atransferrinaemia and GRACILE syndrome, which are considered to be very rare[36,37]. Only type 4 haemochromatosis has an autosomal dominant inheritance while the others are conditions with an autosomal recessive pattern of inheritance[32]. This review discusses only hereditary (or type 1) haemochromatosis.

The forms of secondary haemochromatosis includes those induced by chronic haemolytic anaemia, iron poisoning from excess parenteral iron supplements, and beta-thalassaemia major, sickle cell anaemia, Diamond-Blackfan anaemia or myelodysplastic syndromes that require multiple blood transfusions.

Hereditary haemochromatosis is the most common severe genetic disorder among populations of northern European descent. In 1885, Trousseau was the first to describe haemochromatosis when he observed bronze skin pigmentation in patients with diabetes[38] but it was von Recklinghausen in 1889 who linked iron deposition to the changes seen in this disease and who contributed to its current name[39]. The autosomal recessive inheritance was first noted by Sheldon in 1935[40] and the responsible *HFE* mutation was discovered by Feder *et al.* in 1996[41]. Prior to 2001, units of blood from patients with haemochromatosis were discarded according to the U.S Food and Drug Administration (FDA) unless labelled with the information that the donor had haemochromatosis. Following the demonstration, in 2001, by Sanchez *et al*. that blood donations from haemochromatosis patients are as safe as blood from healthy donors[42], the FDA released guidance for blood banks and recommended not labelling the units with information about the donor's condition. The FDA stated that therapeutic phlebotomy for patients must be done free of charge only if the units are to be entered into the allogeneic blood supply, while it is still acceptable to charge for phlebotomy if the units drawn from a haemochromatosis patient at the facility are discarded. The FDA also insisted that a physical examination be performed and to secure a medical prescription if donations are to be made more frequently than every 8 weeks[43]. Most of the cases are due to two mutations, C282Y and H63D, in the *HFE* gene, which is located on chromosome 6. As their names imply, tyrosine replaces cysteine at the 282nd amino acid in C282Y and aspartic acid replaces histidine at the 63rd amino acid in H63D. The majority of patients with hereditary haemochromatosis are homozygotes for C282Y and less than 5% are C282Y/H63D heterozygotes. A third mutation, S65C, in which cysteine replaces serine at the 65th amino acid, has been implicated in mild forms of hereditary haemochromatosis[44].

The pathophysiology of haemochromatosis is not completely understood but HFE was initially

thought to act on intestinal cells and, when mutated, to cause an increase in iron absorption and subsequently iron overload. Recently, studies done on animals showed that *HFE* mutations can impair the production of another molecule, hepcidin, in the liver and subsequently contribute to iron overload[45]. In normal conditions, the majority of dietary iron is absorbed by duodenal enterocytes and is taken up by the liver. In the liver, iron is used for the synthesis of iron-containing proteins such as cytochromes or it is delivered to the bone marrow and muscles, bound to transferrin, to be incorporated into erythrocyte haemoglobin and used in the synthesis of myoglobin. Remaining iron is kept in the liver bound to the storage proteins ferritin and haemosiderin. Reticuloendothelial cells of the spleen and bone marrow phagocytise senescent erythrocytes, catabolise their haemoglobin and release iron which is returned to the plasma. Duodenal enterocytes, iron-storing hepatocytes, and spleen macrophages release iron into plasma through membrane ferroportin[46]. Hepcidin is a 25-amino acid peptide that inhibits iron transport by binding to ferroportin, which is located on the basolateral surface of enterocytes and the plasma membrane of reticuloendothelial cells. By inhibiting ferroportin, hepcidin maintains iron homeostasis through two mechanisms: it prevents enterocytes from secreting iron into the hepatic portal system, thereby functionally reducing iron absorption, and the release of iron from macrophages. Thus, decreased hepcidin synthesis will cause iron overload[45].

Hereditary haemochromatosis is a multisystem disease that causes excess iron deposition in a variety of organs and tissues, and many patients are asymptomatic or present with signs and symptoms not specific to the disease. Nowadays, because of earlier diagnosis, the classic triad of diabetes, cirrhosis and bronze discolouration of the skin is rarely seen. The most common clinical manifestations are fatigue, lethargy and arthralgia with arthritis of the second and third metacarpophalangeal joints but other joints can be affected. Liver involvement is also common with hepatomegaly, liver cirrhosis and increased risk of hepatocellular carcinoma. Endocrine problems can occur, especially insulin resistance and diabetes, hypothyroidism or hyperthyroidism, and hypogonadotropic hypogonadism, as well as cardiac problems such as congestive heart failure, arrhythmias and pericarditis. Less common findings include skin hyperpigmentation and increased susceptibility to infections such as *Vibrio vulnificus* acquired from seafood followed by *Listeria monocytogenes*, *Yersinia enterocolitica* and *Salmonella enterica*[32].

Early diagnosis is essential and the combination of symptoms, when they are all present, is in itself suggestive of haemochromatosis but the diagnosis is not always easy to infer clinically in young subjects. For example, skin pigmentation, discreet at first, is often interpreted as a normal colour of the skin by the patient because of its chronicity. Other symptoms such as fatigue or arthralgia are both common and not specific in the general population. Often the biological abnormalities will suggest the diagnosis[32].

The initial screening test for haemochromatosis is transferrin saturation, also known as saturation of total iron binding capacity (TIBC); it is most often used in combination with serum ferritin because it lacks specificity. A transferrin saturation >45% with elevated serum ferritin suggests the diagnosis[32,47]. Genetic testing confirms the diagnosis by demonstrating the presence of the mutations C282Y and H63D. Liver biopsy has been used to confirm the diagnosis and to assess cirrhosis in cases of abnormal liver function tests with markedly elevated ferritin; however, with the advent of FerriScan, a test based on magnetic resonance imaging (MRI), liver biopsy has lost its place.

Phlebotomies are the cornerstone therapy for patients with hereditary haemochromatosis but not all signs and symptoms are reversible. It has been difficult to assess the clinical benefit of therapeutic phlebotomy because there are no randomised studies yet that have investigated this subject. A large survey that included 2,851 patients with haemochromatosis to assess the symptoms and the response to therapeutic phlebotomy found that 86% reported that some or all of their symptoms improved with phlebotomy with an average time for improvement of 39±67 weeks, whereas less than 15% of the respondents had a negative opinion about phlebotomy[48]. The most common reported signs or symptoms were extreme fatigue (54.4%), joint pain (43.5%), impotence or loss of libido (25.8%), skin bronzing (25.7%), heart fluttering (23.8%), depression (20.8%) and abdominal pain (20.3%). More than half of the respondents reported improvement of skin bronzing and extreme fatigue (58.8% and 54.4%, respectively), followed by depression (40.8%), abdominal pain (22.3%), impotence or loss of libido (12.7%), joint pain (9.2%) and heart fluttering (6.2%)[48]. Liver function tests and hepatic fibrosis may improve after phlebotomy[49].

Anderson *et al.* demonstrated that not all individuals who are homozygotes for C282Y will develop signs of hereditary haemochromatosis despite having high ferritin and thus will never require phlebotomy[50]. In their study, 23 C282Y homozygotes were identified from 9,174 individuals and were followed for 25 years. All subjects were asymptomatic and did not have a previous diagnosis of haemochromatosis. They had

an average transferrin saturation level above 50% and a mean ferritin level above 400 µg/L. After the 25-year follow up, transferrin saturation and ferritin levels had increased slightly in these patients but none had developed clinically overt haemochromatosis and only one developed subclinical haemochromatosis. Two homozygotes had acute myocardial infarction, one developed diabetes mellitus, and two patients had arthralgia but none had clinical signs of arthritis at physical examination nor skin darkening or hypogonadism. Therapeutic phlebotomy is indicated for symptomatic patients to prevent complications or those who have already developed end-organ damage, with a serum ferritin greater than 300 µg/L for men or post-menopausal women and greater than 200 µg/L for pregnant females[50].

At each phlebotomy session, 450 mL of whole blood are removed which contains approximately 200 to 250 mg of iron. There are no true guidelines regarding the optimal regimen or the end-points of phlebotomy. Some authors recommend performing weekly phlebotomy until the serum ferritin concentration drops to less than 50 ng/mL and transferrin saturation less than 50%[51], While others prefer to continue until they induce an iron deficiency anaemia defined as a haemoglobin concentration of 10 to 12 g/dL, mean cell volume in the lower limit of normal, normal serum ferritin, TIBC >300 µg/dL and transferrin saturation of 10% to 20%[52]. According to Adams *et al*., after reaching these targets, therapeutic phlebotomy should be performed every 2 to 4 months to maintain the serum ferritin between 50 ng/mL and 100 ng/mL[51]. As far as concerns patients with secondary haemochromatosis, if the indication for transfusion that initially led to the iron overload, such as multiple transfusions in bone marrow transplant patients, is no longer present then no long-term maintenance phlebotomy schedule is needed which differentiates this condition from hereditary haemochromatosis.

Erythrocytapheresis is now considered an alternative to phlebotomy: a larger amount of iron can be removed, fewer session are required and the procedures costs the same as phlebotomy[32,53]. For patients who cannot tolerate phlebotomy because of anaemia or other comorbidities, desferrioxamine mesylate or deferasirox is indicated. In patients with cirrhosis or hepatocellular carcinoma, orthotropic liver transplantation is the only available solution[53].

Porphyria cutanea tarda

Porphyria cutanea tarda (PCT) is a rare metabolic disorder caused by uroporphyrinogen decarboxylase deficiency that leads to the accumulation of uroporphyrinogen and highly carboxylated porphyrins in the liver, plasma, urine and sometimes faeces. There are three types of PCT: two familial and one sporadic. Most of the cases are sporadic (80% approximately) and have been associated with several risk factors such as alcohol abuse, hepatitis C, oestrogen use, smoking, hepatic siderosis and human immunodeficiency virus infection[55]. Hepatitis C is one of the most important risk factors; in a recent systematic review and meta-analysis, 50% of PCT patients were found to have hepatitis C infection suggesting an important role in the pathogenesis of PCT although the pathophysiology is still unclear[56,57]. *HFE* gene mutations, especially C282Y homozygosis, has also been found in PCT patients, which explains the iron excess in this disorder, although true haemochromatosis is rare[57].

Clinically, PCT is characterised by chronic blistering skin manifestations that include photosensitivity, increased skin fragility with bullae, erosions, and hyper- or hypo-pigmentation that affects sun-exposed areas of the body; however, these skin manifestations are not specific and they do not confirm the diagnosis. Liver involvement is also common especially in the sporadic form with cirrhosis, fibrosis and increased risk of hepatocellular carcinoma[54]. The diagnosis of PCT requires both clinical and biochemical features. Laboratory investigations include porphyrin chromatographic separation that shows markedly increased uroporphyrins and heptacarboxyl porphyrins in plasma and/or urine, with lesser amounts of penta- and hexa-carboxyl porphyrins. Faecal porphyrins, consisting mainly of isocoproporphyrins, are also increased while the erythrocyte porphyrins are normal[57]. UROD activity analysis with gene sequencing is essential in familial PCT[58].

Liver biopsy is indicated in the event of liver damage demonstrated by markedly increased serum transaminase levels, while skin biopsy is of little benefit because it often only shows sub-epidermal bullae with minimal inflammation[54]. An important aspect of treatment is the avoidance of any risk factors such as alcohol, excessive iron or oestrogen and hepatitis C that can potentially exacerbate the disorder. Therapeutic phlebotomy has long been considered the treatment of choice in most patients with PCT; hydroxychloroquine is the alternative treatment if phlebotomies cannot be tolerated[59]. According to Rocchi *et al.*, 450 mL of whole blood should be removed during each phlebotomy session, with sessions repeated every 2 weeks until the haemoglobin level is below 11 g/dL or until the serum ferritin level is below 20 ng/mL, which is close to the lower limit of normal. Most patients require 6 months to achieve remission but clinical improvement may be noted during the third month after starting phlebotomy[60]. Hydroxychloroquine was found to be superior to phlebotomy in decreasing porphyrin production; however, liver disease was more severe in the hydroxychloroquine group[61]. No

difference was seen between therapeutic phlebotomy and desferrioxamine injection[62].

Skin blistering was the first sign to disappear in patients, at an average of 2 to 3 months and a maximum of 9 months; this was followed by improvement of skin fragility, hypertrichosis and hyper- or hypo-pigmentation while pseudoscleroderma may improve in some patients[59]. Urinary porphyrin levels return to normal but although liver function tests may improve following phlebotomy, the extent of liver damage does not improve[63,64]. Phlebotomy must be stopped after achieving remission or when iron deficiency anaemia occurs; however, relapse may occur during the first 5 years of treatment especially when risk factors are still present, such as excessive alcohol consumption[65].

Other indications

Some authors have suggested that phlebotomy could be a component of the treatment of Alzheimer's disease by reducing body iron load which may be playing an important role in the pathogenesis and progression of the disease; this, however, is a theory and trials are needed to support the hypothesis[66].

In a randomised controlled trial Leo *et al.* observed that iron reduction by phlebotomy can lower the risk of cancer occurrence (38 malignancies *vs* 60 in group in which iron reduction therapy was not used; hazard ratio 0.65; P=0.036). They also observed a lower mortality among phlebotomised group (hazard ratio 0.49; P=0.009)[67]. However, the study had some limitations: it was originally designed as a cardiovascular disease study and not to compare risks of cancer between the two groups. The reported data should, therefore, be considered preliminary and one cannot conclude that lower ferritin and iron levels will reduce the incidence of cancer in unselected healthy individuals because further studies are needed[67].

Iron and serum ferritin have been associated with hyperlipidaemia, diabetes and other cardiovascular risk factors and it was postulated that lowering iron levels could help to reduce these risk factors. Patients with metabolic syndrome who underwent iron reduction by phlebotomy had statistically significant differences in systolic blood pressure, glucose, HbA_{1C}, HDL cholesterol, iron and ferritin compared to controls (P<0.001). LDL cholesterol also decreased but not to a statistically significant degree (P=0.16)[68]. However, the authors indicated that this trial had some limitations. First, the results may not be applicable to patients with metabolic syndrome in general because the definition of metabolic syndrome is not very specific and the sample of patients was small. Second, the study was not blinded. Third, the patients' lifestyle could not be controlled and any lifestyle changes during the study period may have resulted in improvement of the lipid panel and blood pressure. Finally, the study period was only 6 weeks and future trials with longer periods of follow up are needed[68].

Because patients with chronic hepatitis C have difficulty eliminating iron from their bodies, iron toxicity occurs and it was postulated that iron could play a role in the pathogenesis of chronic hepatitis C. Several randomised controlled trials were conducted in order to assess the efficacy of phlebotomy and interferon in the management of chronic hepatitis C compared to interferon alone. One trial demonstrated a better and sustainable viral response in the interferon with phlebotomy groups compared to in the interferon groups (27% *vs* 12%, respectively)[69]. The authors concluded that phlebotomy is a useful adjuvant therapy to interferon in the treatment of chronic hepatitis C[69]. Sartori *et al.* studied the effect of phlebotomy on patients not responding to interferon therapy and showed that phlebotomy can improve histological liver changes in 50% of patients with chronic hepatitis C (P=0.002) with concurrent improvement in liver function tests (P<0.003)[70]. However, this was a retrospective study and the patients receiving the interferon-based therapy were recruited at a time when pegylated interferon and combination therapy were not yet available[70]. Despite all these results, in 2006 the American Gastroenterological Association released guidelines on the management of hepatitis C in which it was stated that phlebotomy cannot currently be recommended as a treatment for patients with chronic hepatitis C[71].

Patients with sickle cell disease (SS or SA) may also benefit from phlebotomy alone or in conjunction with hydroxyurea. Phlebotomy decreases the viscosity of blood by reducing the haemoglobin level and causes a reduction of the mean corpuscular haemoglobin concentration which, in turn, reduces HbS molecule polymerisation in sickle cell disease[72]. In a study of seven children with sickle cell disease suffering from frequent painful crises, Bouchair *et al.* demonstrated that frequent phlebotomies done over a period of 4 years were able to significantly decrease the number of days passed in hospital from 144 days annually to 20, 5, 6 and 1 day, respectively, over the 4 years of observation. The haemoglobin concentration was lowered from an average of 10.7 g/dL before phlebotomy to approximately 9 g/dL after phlebotomy without any adverse events[72]. However, this study included a limited number of patients; additionally, only patients with high haemoglobin are likely to benefit from phlebotomy. Thus, these results do not allow definitive conclusions to be drawn and a larger series of patients would be required to confirm the findings[72]. Another study showed that weekly phlebotomy improved the duration, frequency

and severity of painful crises in 13 patients with sickle cell disease. This was a retrospective study and the authors indicated that the placebo effect of regular phlebotomies can be considerable and, moreover, a degree of amelioration of sickling crises seems to occur with increasing age. However, the beneficial effects of phlebotomy were evident within 3 months after starting the phlebotomies and such effects cannot be attributed to an increase in age[73].

Conclusion

In summary, therapeutic phlebotomy is an essential part of the treatment of various diseases especially those associated with iron overload. In our opinion, it is a safe and cost-effective treatment that should also be considered for use as adjunctive therapy in the treatment of the other disorders discussed in our review.

Keywords: therapeutic phlebotomy, bloodletting, haemochromatosis, polycythaemia vera, porphyria cutanea tarda.

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