

Epidemiology and aetiology of C4–6 disease

Andrew W Bradbury*†

*College of Medical and Dental Sciences, University of Birmingham, Birmingham, UK; †Heart of England NHS Foundation Trust, Birmingham, UK

Abstract

Although our understanding of chronic venous insufficiency (CVI) has improved, many important questions remain unanswered. Ensuring that patients are appropriately referred for specialist assessment and then receive evidence-based, cost-effective treatment continues to be challenging. The lifetime of risk of chronic venous ulceration (CVU) is around 1% with approximately 10% ulcers being open at any one time. The incidence skin changes disease is about 10 times greater (10%). However, many of the studies upon which these estimates are based are old and/or methodologically flawed. There is reason to believe that the incidence, prevalence and characteristics of CVI/CVU may have changed considerably over the last 10–20 years and that future change is likely. Further cross-sectional and longitudinal epidemiological studies are required to establish the size and nature of the health-care need going forward in developed and increasingly developing countries. CVI culminating CVU is primarily the result of sustained ambulatory venous hypertension, which in turn arises from superficial and/or deep venous reflux with or without deep vein obstruction. However, there are many other elements to this complex condition, for example, microvascular dysfunction; calf muscle pump efficiency; dermal inflammation; disordered fibroblast function and matrix production; failure of epithelialization; congenital and acquired thrombophilia; malnutrition, obesity and diet; and bacterial colonization. None of the currently available treatment modalities is entirely satisfactory and novel therapies based upon a clearer understanding of the disease at the psychological, genetic, mechanical, microvascular and microscopic level are required.

Keywords: varicose ulcer; chronic venous insufficiency; CEAP classification; varicose veins

Introduction

The clinical, aetiological, anatomical and pathological (CEAP) classification is now widely used throughout the world to describe lower limb venous disease; the 'C' part of the system refers to the clinical status of the leg with 'C4' being skin changes (e.g. varicose eczema, lipodermatosclerosis, LDS), 'C5' being healed ulceration and 'C6' open ulceration.

Although our understanding of C4–6 disease and how to treat it has undoubtedly improved in recent years,^{1–3} many important questions remain unanswered and, at least in the UK, ensuring that patients are appropriately referred for specialist assessment and then receive evidence-based, cost-effective treatment remains a major challenge.^{4–6}

Correspondence: **Professor A W Bradbury BSc MB ChB Honours MD MBA FRCSEd**, Sampson Gamgee Professor of Vascular Surgery, Birmingham University Department of Vascular Surgery, Flat 5 Netherwood House, Solihull Hospital, Lode Lane, Solihull, West Midlands B91 2JL, UK. Email: andrew.bradbury@btinternet.com

Accepted 21 July 2010

Epidemiology

Many papers, reviews and book chapters on chronic venous insufficiency (CVI) continue to suggest that the lifetime of risk of chronic venous ulceration (CVU) in developed countries is around 1% with approximately 10% of those ulcers being open at any one time.^{7,8} The incidence of C4

disease appears to be about 10 times greater (i.e. around 10%).⁹ However, many of the studies upon which these estimates are based are old and/or methodologically flawed. There is reason to believe that the incidence, prevalence and characteristics of C4–6 disease may have changed considerably over the last 10–20 years and that further change is likely.

For example, given that there appears to be a clear relationship between age and the frequency of venous disease, increasing life-expectancy seems likely to increase the burden of C4–6.¹⁰ Conversely, better prevention and treatment of venous thromboembolic disease might be expected to reduce the number of patients who go on to suffer from the most severe forms of post-thrombotic syndrome.

Aetiology may also have changed. For many years it has been taught that some 80% of all leg ulcers are predominantly due to venous disease. However, in a recent UK survey, only 40% of ulcers were thought to be venous.¹¹ It seems clear that further cross-sectional and longitudinal epidemiological studies of lower limb venous disease are required in order to establish the size and nature of the health-care need going forward in developed, and increasingly developing,^{12,13} countries.

In many respects it is useful to consider C4, C5 and C6 disease together under the term 'skin changes of chronic venous insufficiency' (CVI); thus:

- C4 disease can be usefully thought of as an ulcer that just has not developed yet but probably/possibly will¹⁴ following a triggering event, such as an episode of trauma or perhaps an infected insect bite;
- C6 is an ulcer that will probably heal with appropriate treatment aimed primarily at reducing ambulatory venous hypertension (AVH);
- C5 is a healed ulcer that may well recur unless treatment to minimize AVH is continued.

Aetiology

Macrovascular disease

It is widely accepted that C4–6 disease is due to sustained AVH, which in turn arises from superficial and/or deep venous reflux which can be due to primary valvular insufficiency or can be secondary to deep vein thrombosis (DVT) or superficial vein thrombosis. In a proportion of patients, usually those who have suffered DVT, perhaps triggered by congenital or acquired venous anomalies

(for example compression, atresia and webs), deep vein obstruction is also present.¹⁵

Calf muscle pump

It is important to appreciate that for a given severity of venous reflux and/or obstruction, the severity of the resulting AVH depends on other factors, most importantly the effectiveness of the calf muscle pump.^{16,17} Thus, any condition that impacts upon the calf muscle pump such as immobility, loss of muscle bulk/tone or ankle dysfunction will increase the risks of patients with uncomplicated lower limb venous disease (varicose veins, and C2 and C3 diseases) progressing to C4–6 disease and those with C5/6 disease from healing and remaining healed.^{18–20}

Microvascular disease

The question that remains to be satisfactorily answered is how AVH at the macrovascular level leads to skin injury and CVU at the microvascular level.^{21–23} Many theories have been proposed but none has provided an entirely satisfactory, complete and coherent explanation of the events observed at histopathological, cellular, molecular and biochemical levels.^{7,24–27}

Uncontrolled inflammation

Many studies have observed high levels of inflammatory mediators such as interleukins,²⁸ tumour necrosis factor^{28,29} and C-reactive protein²⁸ in the wound environment. However, whether these are causative of tissue destruction, markers of healing, or merely an epiphenomenon associated with tissue damage and bacterial colonization remains unclear.

When compared with wound fluid from acute wounds, fluid from CVU leads to the production of significantly higher MMP-1 and MMP-3 levels by dermal fibroblasts *in vitro*.³⁰ This had led to a suggestion that soluble mediators in CVU wound fluid lead to an imbalance between matrix metalloproteinases (MMPs) and their inhibitors (tissue inhibitor of metalloproteinases), resulting in excessive proteolysis, tissue destruction, inhibition of granulation and failure of epithelialization.

However, it is also hypothesized that inflammation is 'good' and necessary for healing. Thus, compression leading to CVU healing has been associated with a pro-inflammatory environment characterized by elevated levels of IL-1,

IL-6, IL-12p40, granulocyte macrophage colony-stimulating factor (GM-CSF), IL-1 receptor antagonist, IFN- γ and MMPs.^{31,32}

These types of basic science studies raise the possibility that treatments aimed at manipulating the inflammatory milieu in a 'helpful' way might promote CVU healing.^{33,34}

For example, GM-CSF treatment of CVU has been shown to:

- Increase vascular endothelial growth factor transcription in the ulcer bed;
- Promote angiogenesis leading to increased blood vessel density.³⁵

However, determining which molecular signals in CVU wound fluid and the systemic circulation are important and which simply represent 'noise' from what is a very complex biological environment remains a significant challenge.³⁶

Fibroblast senescence and disordered extracellular matrix production

It is widely accepted that the longer a CVU has been present, the harder it is to heal and to keep healed. The question is whether these 'hard to heal' ulcers are different from the outset or whether they become so as time passes. As might be expected from such a complex and, as yet, still poorly understood phenomenon as wound healing and remodeling, studies of chronic wounds in general suggest that healing might be impaired through a number of different mechanisms acting at various points along the healing pathway.

One hypothesis that has received quite a lot of attention is that the continuing, unresolved inflammation found in chronic wounds leads to 'stress-induced', premature senescence of fibroblasts such that the connective tissue scaffold upon which wound healing takes place fails to develop.³⁷ It is also possible that senescent fibroblasts release various factors and a type of 'degraded' extracellular matrix that further inhibits wound healing. The accumulation of more than 15% senescent fibroblasts within a wound has been described as a threshold beyond which wounds become hard to heal. The ratio of senescent to non-senescent cells may, therefore, be critical to determining response to treatment. Furthermore, adjunctive therapies that modulate this ratio in favour of non-senescent cells may enhance healing rates.

Specifically, it has been hypothesized that commercially available tissue-engineered dermal

replacements such as Dermagraft (Smith and Nephew, www.smith-nephew.com) can 'kick start' chronic wounds by donating non-senescent fibroblasts to the wound environment, leading to the release of growth factors that will reverse the anti-proliferative activity of chronic wound exudate. Unfortunately, despite the expenditure of hundreds of millions of dollars on R&D, a lack of clinical and cost-effectiveness has meant that these types of products have not found their way into everyday clinical use for the treatment of CVU, at least in the UK (<http://www.apligraf.com>).

The regulation of extracellular matrix is complex, and the mechanisms underlying the abnormalities observed in patients with C4–6 disease are not well understood. However, it has been suggested that disordered TGF- β (1) signalling may be implicated and that therapeutic molecular targets aimed at this pathway might enhance matrix contraction and thereby improve venous ulcer wound healing.³⁸ Other workers have suggested that treatment aimed at altering the MAPKp38 pathway *in vivo* through the use of either growth factors or cytokine inhibitors might improve fibroblast proliferation and thus CVU healing.³⁹

While failure of wound healing suggests fibroblast failure, paradoxically, somewhat akin to the need for 'balanced' inflammation,⁴⁰ it is clear that C4–6 disease is characterized by excessive fibrosis and reduced skin elasticity. These changes may make the skin more prone to shearing injury, reduce perfusion and so adversely affect macro- and microvascular function. Perhaps not surprisingly given the clinical appearances, skin affected by LDS has been found to contain an increased proportion of fibroblasts expressing procollagen type I mRNA (COL1A1) and undergoing proliferation. Interestingly, this has also been detected in the skin of patients with venous disease prior to the development of frank skin changes.⁴¹

Failure of epithelialization

Failure of epithelialization despite an apparently healthy, well-vascularized base of granulation tissue is a common finding in CVU. Biopsies from the margins of non-healing CVU often reveal a hyperkeratotic and hyperproliferative epidermis, suggesting incomplete activation and differentiation of keratinocytes. In keeping with these clinical and histopathological features, such epidermis exhibits abnormal expression of early (keratins K1/K10) and late (filaggrin, involucrin and transglutaminase 1) differentiation and activation

(K6/K16/K17) markers, as well as deregulated desmosomal and tight junction components.⁴² As with fibroblasts, the molecular mechanisms underlying keratinocyte failure remain unclear and are the subject of ongoing investigations.^{43,44}

Thrombophilia

Some of the most severe and intractable CVU occur as part of the postphlebotic syndrome, especially when it develops following proximal and recurrent DVT. In a recent study of CVU patients screened for the factor V Leiden and prothrombin 20210A mutations; antithrombin, protein C and S deficiencies; antiphospholipid antibodies; hyperhomocysteinaemia (HHcy) and factor VIII, IX and XI levels, the overall prevalence of single and multiple thrombophilias were found to be significantly higher than in age- and sex-matched controls without venous disease.⁴⁵ Other workers have also found HHcy, an independent risk factor for venous thrombosis, in patients with C4–6 when compared with healthy controls.⁴⁶ These data support the hypothesis that congenital and acquired thrombophilia may predispose to the development of CVU through the increased occurrence of clinical and sub-clinical thrombosis in both the deep and the superficial venous systems.⁴⁷ Although HHcy can be treated by selected vitamin supplementation, even in patients with normal serum vitamin concentrations, it remains to be seen whether such treatment will alter the natural history of C4–6 disease.⁴⁸ Similarly, it is unknown whether long-term anticoagulation in patients at risk of DVT will reduce the burden of CVU.

Malnutrition

For many years it has been thought that malnutrition and a diet deficient in certain important elements, such as zinc and various vitamins, might be implicated in the development and chronicity of CVU. Two recent studies have confirmed that the prevalence of protein deficiency and malnutrition in patients with CVU is high and associated with poor healing.^{49,50} However, the relationship between diet and CVU is complex because obesity also predisposes to lower limb venous disease and everyday clinical experience indicates that many patients with intractable CVU appear to be significantly overweight.

In a recent study of obese CVU patients, nutritional status was evaluated using anthropometric measurements, nutrient analysis from a three-day

dietary intake log, serum albumin, vitamins A and C, and zinc levels. Wound severity was assessed using the leg ulcer measurement tool (LUMT). When compared with recommended daily intake levels, dietary nutrient intake was suboptimal for protein, vitamin C and zinc. Serum levels were below normal for at least one of these nutrients in six of eight patients. An inverse relationship was found between LUMT score and serum vitamin A levels and a positive correlation was observed between LUMT score and serum vitamin C. No clear relationships were shown among serum zinc, albumin and LUMT scores.

Thus, obese patients with CVU may exhibit nutritional deficits that may be related to the severity of their ulcer disease.⁵¹ Further work is required to confirm these findings and to determine whether diet modification can alter the natural history of C4–6 disease.

Bacteria

It is widely taught that bacterial colonization of ulcer (common) as opposed to infection (uncommon) plays little role in the pathogenesis of CVU and most authorities (at least in the UK) believe that antibiotics, either topical or systemic, and antiseptics have little role to play in treatment.⁵² However, it has been proposed that failure of healing in CVU is at least in part caused by inefficient eradication of infecting, opportunistic pathogens, a situation that has been described as reminiscent of chronic *Pseudomonas aeruginosa* infections found in patients with cystic fibrosis. Sections from chronic wounds studied by fluorescence *in situ* hybridization have been found to contain distinct microcolonies – the basal structures of bacterial biofilms. Furthermore, it is suggested that these *P. aeruginosa* biofilms create a shielding mechanism that offers protection from the phagocytic activity of polymorphonuclear leukocytes⁵³ and that bacteria located in the deeper regions of CVU keep the wound arrested in a stage dominated by damaging inflammatory processes.⁵⁴ While these hypotheses require further testing, it does raise the possibility that novel therapeutic measures aimed at eradicating bacteria, such as bacteriophages against *P. aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*, might increase the speed and quality of healing.⁵⁵

Conclusions

Much about the epidemiology and aetiology of C4–6 disease remains unknown or uncertain.^{56–60} None

of the currently available treatment modalities is entirely satisfactory^{20,61} and novel therapies based upon a clearer understanding of the disease at the psychological, genetic, mechanical, microvascular and microscopic level are required.^{27,34,62–72}

Sources of financial support: None.

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