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Panton-Valentine Leukocidin Osteomyelitis in Children – A Growing Threat

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Short Introduction

Panton-Valentine leukocidin (PVL) producing *Staphylococcus aureus* causes a potentially fatal osteomyelitis in children that is increasing in incidence. It has multiple complications that differentiate it from PVL-negative disease including multiple abscesses, DVT and fulminant sepsis. We present a review unifying the literature concerning this emerging threat that is currently under-recognised.

Introduction

Osteomyelitis is a common paediatric infection encountered in orthopaedics. *Staphylococcus aureus* (*S. aureus*) remains the commonest causative pathogen encountered in 70-90% of cases (Bocchini et al, 2006). *S. aureus* produces a number of toxins including the Panton-Valentine leukocidin (PVL) toxin, first described in 1932. In the United Kingdom (UK), approximately 1-5% of clinical isolates of *S. aureus* produce PVL toxin, with production in both methicillin-susceptible *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) in the UK (Shallcross et al, 2013). Within orthopaedics, PVL producing *S. aureus* (PVL-SA) has been associated with severe osteomyelitis and complications e.g. pulmonary infections, that can be fatal (Shallcross et al, 2013, Rafai et al, 2013).

We present a clinical review unifying the literature concerning PVL-SA osteomyelitis which is likely under-recognised due to lack of awareness and infrequent diagnostic testing.

Structure of PVL and Leukocytotoxicity

PVL is a bi-component cytotoxin produced by certain strains of *S. aureus*. The detection of PVL-SA is either by PCR detection of the gene or by toxin assays. Most *S. aureus* that possess the *pvl* gene produces toxic concentrations of PVL in-vitro (90%) (Badiou et al, 2010).

PVL belongs to a family of synergo-hymenotropic (assemble to form pores) proteins that are leukocytotoxic. PVL protein subunits assemble in white cell membranes (particularly monocytes and macrophages) to form a central pore through which cell contents can leak (Szmigielski et al, 1999, Kaneko and Kamio, 2004). The resulting apoptosis and necrosis (Figure 1) is able to compromise the immune response of the infected micro-environment and facilitate the spread of bacteria (Genestier et al, 2005). The assembled PVL protein can also act as a super-antigen and therefore trigger a massive immune response (Deresinski, 2005).

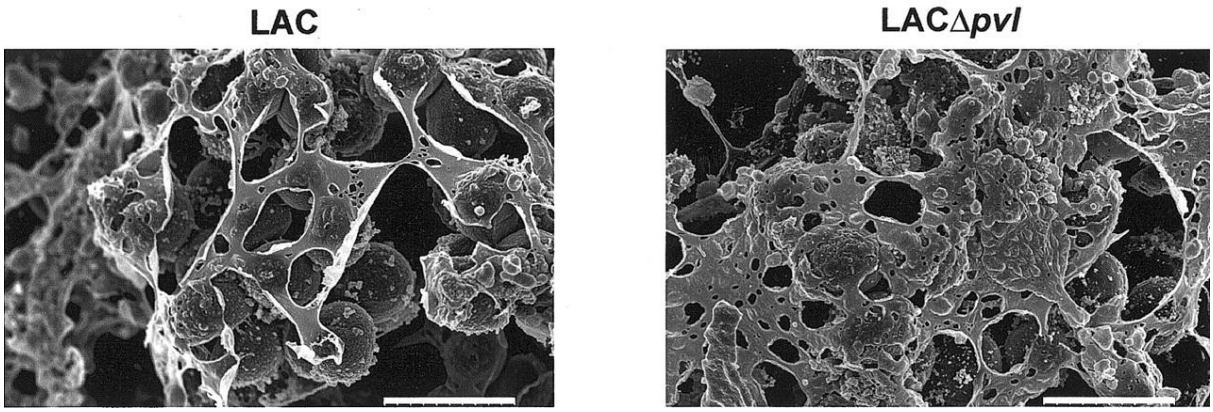

Figure 1
Ultrastructural analysis of necrosis of human polymorphonuclear leukocytes infected with PVL-negative MRSA (LAC) and PVL-MRSA (LACΔpvl). Scanning electron microscopy. Bar in left panel, 1.67 μm; bar in right panel, 1.48 μm.
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Epidemiology of PVL Osteomyelitis

In developed countries, the incidence of paediatric osteomyelitis is between 1.9-13/100,000 and the commonest causative organism is *S. aureus* (Dartnell et al, 2012). Due to a paucity of literature evidence, the true incidence of PVL-SA osteomyelitis is not known. According to the Health Protection Agency of the UK (HPA), the overall incidence of PVL-SA infections remain low but with evidence of annual increase, possibly due to increased awareness and more samples referred for testing (Health Protection Agency, 2008).

In UK, the PVL toxin is secreted by 1-2% of all S. aureus isolates and around 5% of isolates from established skin and soft tissue infection (Shallcross et al, 2013). The majority of these isolates cause skin and superficial soft tissue infections and far fewer musculoskeletal infections (Holmes et al, 2005, Shallcross et al, 2013).

The prevalence of PVL-SA in osteomyelitis in the UK is not known. In the United States (US), PVL-SA accounts for up to two-thirds of *S. aureus* related paediatric osteomyelitis (Bocchini et al, 2006, Sdougkos et al, 2007). It is attributed to the USA-300 clone of MRSA which is the most common in the US. The majority of the current literature on PVL-SA originates from the US, giving the impression that PVL-SA is associated with the reported severe disease associated with USA-300 MRSA. There is however uncertainty if USA-300 virulence results from PVL or other virulence factors that cause severe disease. Within the United Kingdom and Europe, the USA-300 strain is infrequently reported, and over half of PVL-SA strains are associated with MSSA (Holmes et al, 2005).

In a large Australian series of 478 patients, PVL-SA was more common in MRSA than MSSA (54% vs 40%) infections (Tong et al, 2010). Tong et al. concluded PVL-SA causes clinically distinct infections compared to PVL-negative strains and emphasised that PVL-SA is present in MSSA but under-recognised. In the context of paediatric osteomyelitis, the PVL strain is reported in 9-21% of MSSA infections whereas in comparison 87-100% of MRSA related infection had PVL toxin confirmed (Bocchini et al, 2006, Martinez-Aguilar et al, 2004). Interestingly these studies also reported that PVL-SA disease was associated with community acquired infection in younger age groups presenting with overwhelming sepsis.

PVL in Osteomyelitis

Clinical presentation: A recent meta-analysis suggested that PVL-SA is a more common cause of skin and soft tissue infections than deeper musculoskeletal infections. Children, however, with PVL-SA musculoskeletal infection *may* be at an increased risk of morbidity (Shallcross et al, 2013). The presenting symptoms of PVL-SA osteomyelitis are generally similar to PVL-negative cases – however, disease progression is reported to be more aggressive and rapid with severe presentations e.g. severe sepsis, more severe pain, a greater inability to weight bear and/or swelling of the affected limb (Mitchell et al, 2007). The location of osteomyelitis is similar, with over 60% of infections affecting the femur, tibia or fibula in both PVL-positive and PVL-negative disease (Bocchini et al, 2006).

Clinical severity: PVL-SA osteomyelitis can be multifocal with multiple bones involved. Patients are also significantly more likely to present with large sub-periosteal abscesses (risk ratio 3.90, p=0.25, Figure 3), multiple bony abscesses (Figure 4) and associated myositis/pyomyositis (risk ratio 3.22, p=0.18, Figure 5) diagnosed on magnetic resonance imaging (MRI) scan (Figure 4). Comparatively, PVL-negative osteomyelitis usually occurs with a single focus of infection without peri-focal involvement (Bocchini et al, 2006, Dohin et al, 2007, Ceroni et al, 2012).

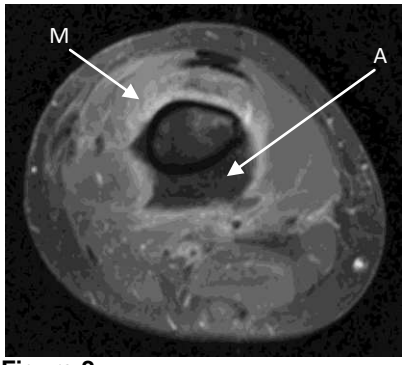

Figure 2
Axial MRI sequence of a femur infected with PVL-SA osteomyelitis.
Note the large subperiosteal abscess (A) and extensive high signal in the surrounding muscles indicating myositis (M)

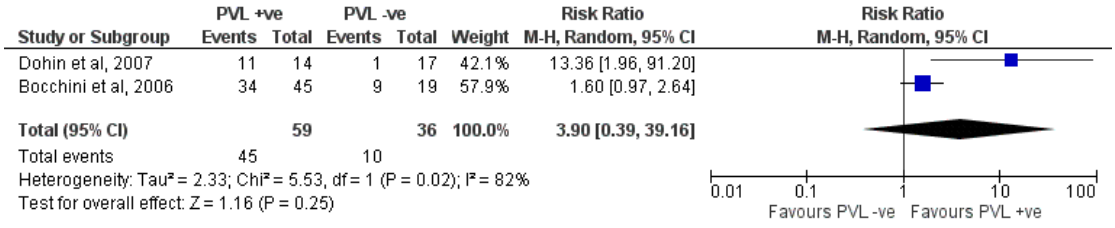

Figure 3
Forest Plot: Risk Ratios of Subperiosteal Abscess

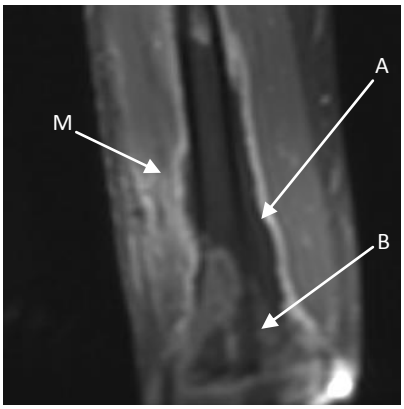

Figure 4
Coronal MRI sequence of a femur infected with PVL-SA osteomyelitis.
Subperiosteal abscess (A), bony abscess (B) and myositis (M)

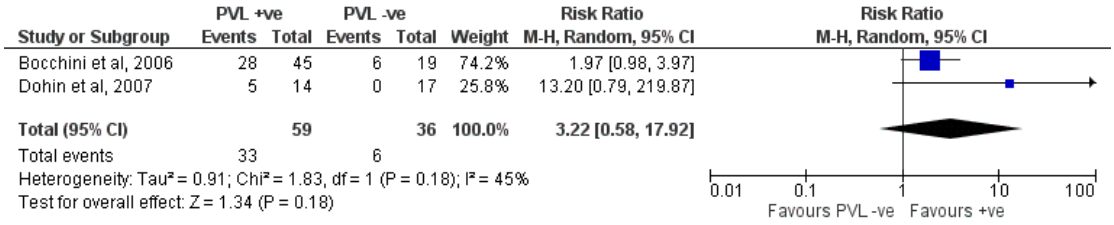

Figure 5
Forest plot: Risk Ratios of Myositis/Pyomyositis

Studies have reported that PVL-SA osteomyelitis cases have a more severe disease course. Martinez-Aguilar et al. (2004) reported in their series of 59 children with *S. aureus* musculoskeletal infections (81% osteomyelitis), that PVL-SA was significantly associated with longer febrile days and more complications (10 *vs* 0). Similarly Bocchini et al. (2006) and Sdougkos et al. (2007) have reported in *S. aureus* osteomyelitis that those with PVL-SA had higher biochemical inflammatory markers at presentation and at their peaks and take longer to normalise. Bocchini et al. (2006) reported in 89 children that blood cultures were more likely to be positive for *S. aureus* (67.2% vs 19.2%) and that they were more likely to require ICU admission. These patients are also likely to have a longer illness course, with more febrile days and a longer inpatient stay (Dohin et al, 2007). Diagnostic ambiguity is therefore less likely in PVL-SA osteomyelitis compared to PVL negative cases, owing to a more severe clinical presentation with much higher inflammatory markers and positive blood cultures. Such a clinical picture should prompt the clinician to consider assessing for PVL-SA associated complications e.g. deep vein thrombosis (DVT) and multifocal infection.

Osteomyelitis caused by PVL-SA has resulted in systemic complications including overwhelming sepsis and septic shock (Mitchell et al, 2007). This is usually a disseminated disease process and often poses clinical difficulty in differentiating from those with a primary bony infection. Complications include multifocal infections including infective endocarditis,

cerebral infarcts, deep vein thromboses (DVTs) and rhabdomyolysis with acute kidney injury requiring renal replacement therapy. Multi-organ dysfunction leading to mortality has been reported in adults (Rafai et al, 2013). A rare reported local complication is spontaneous tibio-talar fusion two months following PVL-SA osteomyelitis of the distal tibial diaphysis (Ceroni et al, 2012). The authors suggested that the pathophysiology of the auto-arthrodesis is linked to higher levels of metallo-proteinases and collagen degrading enzymes that are found in the massive immune response following septic arthritis by PVL-SA. Long term sequelae are therefore common following PVL-SA osteomyelitis and require a longer duration of follow up as compared to PVL-negative osteomyelitis (Dohin et al, 2007).

Pulmonary infection is an important systemic complication of PVL-SA osteomyelitis. Broadly speaking, pulmonary infection in osteomyelitis has been shown to be related to DVT, probably through septic emboli (Vander Have et al, 2009, Gonzalez et al, 2006). Indicators of pulmonary involvement in PVL-SA infection include haemoptysis, abnormal chest radiograph, multi-lobar involvement and leukopenia (Mitchell et al, 2007, Gonzalez et al, 2005). Pulmonary infection with PVL-SA is a very poor predictor of outcome, with a reported mortality of 75.8% (Khanafer et al, 2013).

Venous Thromboembolism

The presence of DVT is one of the hallmark features of PVL-SA osteomyelitis. Primary venous thromboembolism (VTE) in children is rare. Annual incidence is 0.06-0.07/10,000 compared to 2-7/10,000 in adults (Lachambre et al, 2005). The incidence in association with acute osteomyelitis, however, is increasing and is present in around 9% of paediatric cases with osteomyelitis (Bouchoucha et al, 2010). Such patients with DVT (Figure 4) are more likely to be infected with MRSA.

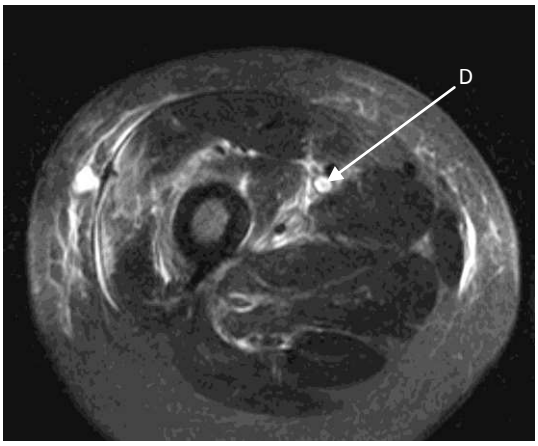

Figure 6
Axial MRI sequence of a femur infected with PVL-SA osteomyelitis showing multiple complications including DVT (D) in the femoral vein

Gonzalez et al. (2006) reported nine cases of DVT associated with acute haematogenous osteomyelitis. Seven of these (78%) were caused by PVL-MRSA. Similarly Dohin et al. (2007) found that three of their 14 paediatric PVL-SA osteomyelitis cases developed a DVT in comparison to none of their 17 patients with PVL-negative osteomyelitis (21% vs 0%, p=0.08). These findings have been echoed in other studies which have shown a strong association between paediatric PVL-SA musculoskeletal infections and the risk of developing DVT (Martinez-Aguilar et al, 2004, Obando et al, 2011). It should, however, be borne in mind that the true incidence in the context of PVL osteomyelitis is likely much higher than reported as previous studies have not routinely investigated for this complication. Although the exact pathophysiology of this is not well understood, it may be linked to the greater systemic response found in PVL osteomyelitis in relation with a massive local inflammatory response since DVTs are invariably found adjacent or proximal to the site of osteomyelitis (Gonzalez et al, 2006).

Patients with osteomyelitis and DVT are likely to have a much greater systemic response to their illness with more prominent local signs, a higher temperature, longer duration of pyrexia, higher ESR and CRP levels and a positive blood culture. Surgery is likewise more likely to be required for abscess drainage in these patients (Hollmig et al, 2007, Bouchoucha et al, 2010).

DVT is more commonly seen in patients with DVT in PVL infection than with PVL negative *S. aureus* infection (risk ratio=8.07, p=0.04) (Figure 7).

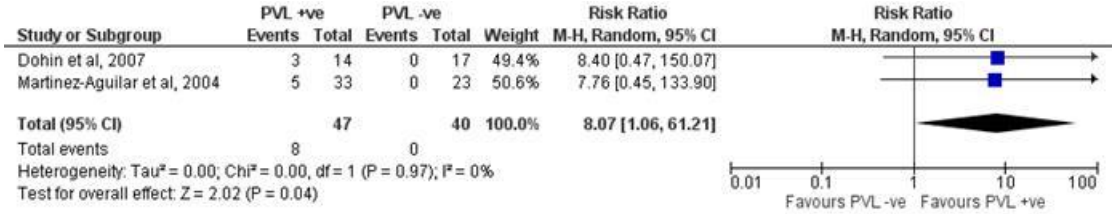

Figure 7
Forest plot: Risk Ratios of DVT

Investigations

Whilst PVL-negative osteomyelitis has a comparatively more insidious onset, PVL-SA osteomyelitis tends to present rapidly. As discussed above, inflammatory markers are higher in PVL-SA osteomyelitis. Blood cultures are frequently positive for *S. aureus* in PVL-SA disease and stay positive for longer than PVL-negative disease (Martinez-Aguilar et al, 2004). This may reflect an intravascular source of some of these infections, such as infected DVTs. Current British Society for Children's Orthopaedic Surgery (BSCOS) recommendation is to delay the commencement of antibiotics until specimens of suspicious tissue are obtained apart from in the very unwell child where antibiotic treatment should start immediately. Deep tissue samples or synovial fluid (in septic joints) will isolate organisms in up to 80% of cases (British Society for Children's Orthopaedic Surgery, 2013). The clinical utility of PVL testing is uncertain, as disease severity should prompt investigations for complications of osteomyelitis. Public Health England currently do not specifically request *S. aureus* isolates from osteomyelitis cases are sent for PVL testing. They do suggest necrotising skin/pulmonary infections, and those with recurrent abscesses or where an outbreak is suspected are considered for PVL testing. Conventional bacterial culture does not differentiate between PVL-positive and PVL-negative SA. Diagnosis can be made by detection of the *pvl* gene using polymerase-chain reaction analysis of bacterial DNA (Bocchini et al, 2006). Newer techniques allowing rapid detection include enzyme-linked immunosorbent assay and immunochromatographic tests for the PVL toxin (Badiou et al, 2010).

Given PVL testing is often a reference test, or may not be completed according to national guidelines, it will often be the case that PVL status is not known. The evidence presented though suggests those with severe presentations of osteomyelitis should be considered as possibly being PVL positive and investigated as such.

Early plain radiographic findings may be subtle or absent and therefore other modalities of imaging such as MRI are invaluable in establishing the focus/foci of infection and any local complications including incidental findings of DVT. As the abscesses may recur, repeat MRI scan is indicated if the clinical picture does not improve despite drainage of pus and appropriate antibiotic therapy. Pulmonary complications can include emboli, which are not infrequently septic and imaging of the chest needs therefore to be targeted to the clinical presentation. Gonzalez et al. (2005) reported that in a series of 113 children with invasive *S. aureus* infections, abnormal chest x-ray findings were present in 51 of 80 patients with PVL-SA and in a multivariate analysis the presence of PVL-SA was significantly associated with secondary pneumonia in children with an abnormal chest x-ray.

The role of routine investigation for DVT in children with osteomyelitis is not yet established in literature. Hollmig et al. (2007) recommended that all children with osteomyelitis should have diagnostic venous imaging performed to assess for DVT. Gonzalez et al. (2006) suggested using Duplex-Doppler ultrasound venography to assess for DVT. However, this technique may be unreliable as surrounding myositis and oedema may alter venous blood flow. Multiple treatment options for DVT in children with osteomyelitis are described including low-molecular-weight heparin, intravenous heparin and intra-caval filters for severe cases (Gonzalez et al, 2006). The ideal treatment, however, is not yet established.

Management

Surgical debridement and washout remains an important aspect of management in most PVL-SA bone infections to gain local control of infection and multiple procedures may be necessary (Dohin et al, 2007, Bocchini et al, 2006, Ceroni et al, 2012). This is in contrast to recent guidelines by the BSCOS which states that antibiotic therapy is sufficient and surgery does not confer any additional benefits in most cases (British Society for Children's Orthopaedic Surgery, 2013). The aim of drainage is not only to halt the local infective process, but also to remove the “glutinous pus” that makes local delivery of antibiotics difficult (Mitchell et al, 2007). As the pus is thick and difficult to drain arthroscopically, an open approach may be preferable if joint washouts are necessary. Dohin et al. (2007) and Bocchini et al. (2006) both reported that patients with PVL-SA osteomyelitis were more likely to require surgical exploration and drainage. The former reported that 71% of these patients required surgical procedures, often multiple (range 1-5), while only 17% of non-PVL osteomyelitis patients required a single surgical procedure.

Surgical techniques beyond simple drainage and debridement have been described. The use of a well vascularised inter-positional muscular flap such as that from the gastrocnemius in cases of tibial infection has been advocated to allow adequate soft tissue coverage

following extensive bone and soft tissue debridement. The use of muscle interposition flaps to cover debrided bony defects has previously been used in chronic osteomyelitis (Smith et al, 2006) but has also proven useful in treating acute PVL-SA osteomyelitis.

Targeted antibiotic therapy is the key to successful treatment following drainage of any associated abscess. Currently, there is a paucity of literature comparing the effectiveness of antibiotic regimens for PVL osteomyelitis. As the majority of cases of osteomyelitis are caused by MSSA, flucloxacillin is a common empirical first line antibiotic employed in most institutions. However, increasing numbers of bone infections are now caused by MRSA and this only becomes evident after appropriate blood or tissue culture results are available. In such instances, antibiotics with activity against MRSA such as vancomycin are commenced. PVL-SA is linked to both MRSA and MSSA, however, and therefore first line antibiotics may not necessarily have significant action against the micro-organism.

Clindamycin, rifampicin, linezolid and fusidic acid all decrease PVL production (Dumitrescu et al, 2008). In sub-inhibitory concentrations, oxacillin has been shown to increase PVL production by 250% in-vitro (Dumitrescu et al, 2008). There are however no clinical studies demonstrating this in-vivo. Combination therapy of oxacillin and clindamycin or rifampicin or linezolid all inhibited PVL production. Isolated case reports of using other antibiotics such as daptomycin effectively have also been published (Erturan et al, 2012).

Current practice in England is based around guidance from the BSCOS and antibiotics with anti-toxin effects are recommended. For proven PVL-SA infection clindamycin is used as first line therapy with the addition of vancomycin in methicillin and clinidamycin resistant cases. Linezolid has also shown to be useful in cases where these antibiotics do not show clinical improvement (British Society for Children's Orthopaedic Surgery, 2013). Ultimately, however, the most effective antibiotic regimen depends upon the sensitivity of the cultured micro-organism and oral antibiotics usually have to be continued on a long-term basis until clinical and biochemical markers of the infection settle. PVL-SA positive patients are likely to have recurrent abscesses and/or recurrence of bone infection and therefore require a longer follow up. Another important reason to follow up is presence of associated DVT. The ideal duration of follow up after discharge from hospital is not established, however, one study reported that thrombus resolved after a mean of 11 weeks (Hollmig et al, 2007). From the authors' previous experience with this condition, serial MRI scans were invaluable in assessing disease progression and identifying late complications e.g. recurrent abscess.

Conclusion

PVL-SA has can cause severe osteomyelitis that presents acutely, deteriorates quickly, can requires longer inpatient care and has multiple short and long term sequelae than non PVL-SA osteomyelitis. Well documented clinical associations of PVL osteomyelitis include sub-periosteal abscesses, myositis/pyomyositis, DVT and potentially fulminant pulmonary sepsis.

At presentation, due to a severe clinical picture, there is normally minimal diagnostic ambiguity that a patient with PVL-SA osteomyelitis has an aggressive infective process. Expediting confirmation of diagnosis and sensitivities will ensure early use of appropriate antibiotic regimen.

If clinical suspicion of PVL-SA exists (effectively in patients with severe osteomyelitis), empirical antibiotic treatment should be administered early with MRI of the affected site(s). An effective treatment regimen requires drainage of associated abscesses. As the abscesses/foci of infection may be multiple and/or serial, the use of serial MRI scans is invaluable. MRI scanning has the added advantage of identifying local extension of infection and DVTs.

The ideal antibiotic regimen will vary with local prevalence of strains and sensitivities. There is a current effort to develop an anti-PVL toxin with limited success in-vitro (Libert et al, 2009), and this approach may prove beneficial in combating this emerging threat as more sophisticated treatments become available.

Current literature on PVL-SA musculoskeletal infections consist mostly of retrospective cohort studies and case series. Long term epidemiological, diagnostic and therapeutic studies relating to the role of PVL-SA in osteomyelitis are needed in the UK population. Awareness of PVL-SA bone infections and its related sequelae is of paramount importance amongst clinicians treating cases of acute osteomyelitis in a paediatric population.

Conflicts of Interest

None declared

Keywords: Panton-Valentine leukocidin, paediatric, osteomyelitis, deep vein thrombosis, complications, treatment

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Review Manager (RevMan) [Computer program]. Version 5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014. The above freeware software application was used to create the forest plots used in this article.

References

Badiou C, Dumitrescu O, George N, Forbes AR, Drougka E, Chan KS, Ramdani-Bouguessa N, Meugnier H, Bes M, Vandenesch F, Etienne J, Hsu LY, Tazir M, Spiliopoulou I, Nimmo GR, Hulten KG & Lina G (2010) Rapid detection of Staphylococcus aureus Panton-Valentine leukocidin in clinical specimens by enzyme-linked immunosorbent assay and immunochromatographic tests. *J Clin Microbiol* **48**(4): 1384-90.

Bocchini CE, Hulten KG, Mason EO, Jr., Gonzalez BE, Hammerman WA & Kaplan SL (2006) Panton-Valentine leukocidin genes are associated with enhanced inflammatory response and local disease in acute hematogenous Staphylococcus aureus osteomyelitis in children. *Pediatrics* **117**(2): 433-40.

Bouchoucha S, Benghachame F, Trifa M, Saied W, Douira W, Nessib MN & Ghachem MB (2010) Deep venous thrombosis associated with acute hematogenous osteomyelitis in children. *Orthop Traumatol Surg Res* **96**(8): 890-3.

British Society for Children's Orthopaedic Surgery (2013) The management of acute bone and joint infection in childhood - a guide to good practice.

Ceroni D, de la Llana RA, Zand T, Lamah L, Dominguez D, De Coulon G & Dubois-Ferriere V (2012) Spontaneous tibiotalar arthrodesis as a complication of acute tibial osteomyelitis due to Panton-Valentine leukocidin-producing Staphylococcus aureus: a case report. *J Med Case Rep* **6**(1): 202.

Dartnell J, Ramachandran M & Katchburian M (2012) Haematogenous acute and subacute paediatric osteomyelitis: a systematic review of the literature. *J Bone Joint Surg Br* **94**(5): 584-95.

Deresinski S (2005) Methicillin-resistant Staphylococcus aureus: an evolutionary, epidemiologic, and therapeutic odyssey. *Clin Infect Dis* **40**(4): 562-73.

Dohin B, Gillet Y, Kohler R, Lina G, Vandenesch F, Vanhems P, Floret D & Etienne J (2007) Pediatric bone and joint infections caused by Panton-Valentine leukocidin-positive Staphylococcus aureus. *Pediatr Infect Dis J* **26**(11): 1042-8.

Dumitrescu O, Badiou C, Bes M, Reverdy ME, Vandenesch F, Etienne J & Lina G (2008) Effect of antibiotics, alone and in combination, on Panton-Valentine leukocidin production by a Staphylococcus aureus reference strain. *Clin Microbiol Infect* **14**(4): 384-8.

Erturan G, Holme H, Smith R, Dodds R & Iyer S (2012) Successful use of daptomycin in Panton-Valentine leucocidin positive Staphylococcus aureus paediatric osteomyelitis. *Int J Surg Case Rep* **3**(7): 238-41.

Genestier AL, Michallet MC, Prevost G, Bellot G, Chalabreysse L, Peyrol S, Thivolet F, Etienne J, Lina G, Vallette FM, Vandenesch F & Genestier L (2005) Staphylococcus aureus Panton-Valentine leukocidin directly targets mitochondria and induces Bax-independent apoptosis of human neutrophils. *J Clin Invest* **115**(11): 3117-27.

Gonzalez BE, Hulten KG, Dishop MK, Lamberth LB, Hammerman WA, Mason EO, Jr. & Kaplan SL (2005) Pulmonary manifestations in children with invasive community-acquired Staphylococcus aureus infection. *Clin Infect Dis* **41**(5): 583-90.

Gonzalez BE, Teruya J, Mahoney DH, Jr., Hulten KG, Edwards R, Lamberth LB, Hammerman WA, Mason EO, Jr. & Kaplan SL (2006) Venous thrombosis associated with staphylococcal osteomyelitis in children. *Pediatrics* **117**(5): 1673-9.

Health Protection Agency (2008) Guidance on the diagnosis and management of PVL-associated Staphylococcus aureus infections (PVL-SA) in England.

Hollmig ST, Copley LA, Browne RH, Grande LM & Wilson PL (2007) Deep venous thrombosis associated with osteomyelitis in children. *J Bone Joint Surg Am* **89**(7): 1517-23.

Holmes A, Ganner M, McGuane S, Pitt TL, Cookson BD & Kearns AM (2005) Staphylococcus aureus isolates carrying Panton-Valentine leucocidin genes in England and Wales: frequency, characterization, and association with clinical disease. *J Clin Microbiol* **43**(5): 2384-90.

Kaneko J & Kamio Y (2004) Bacterial two-component and hetero-heptameric pore-forming cytolytic toxins: structures, pore-forming mechanism, and organization of the genes. *Biosci Biotechnol Biochem* **68**(5): 981-1003.

Khanafer N, Sicot N, Vanhems P, Dumitrescu O, Meyssonier V, Tristan A, Bes M, Lina G, Vandenesch F, Gillet Y & Etienne J (2013) Severe leukopenia in Staphylococcus aureus-necrotizing, community-acquired pneumonia: risk factors and impact on survival. *BMC Infect Dis* **13**(359.

Lachambre G, Proulle V, Bader-Meunier B & Dreyfus M (2005) [Misdiagnosis of venous thrombosis in childhood]. *Arch Pediatr* **12**(2): 180-2.

Libert N, Batjom E, Cirodde A, de Rudnicki S, Grasser L, Borne M & Brinquin L (2009) [Antitoxin treatments for necrotizing pneumonia due to Panton-Valentine leukocidin-secreting Staphylococcus aureus]. *Med Mal Infect* **39**(1): 14-20.

Martinez-Aguilar G, Avalos-Mishaan A, Hulten K, Hammerman W, Mason EO, Jr. & Kaplan SL (2004) Community-acquired, methicillin-resistant and methicillin-susceptible Staphylococcus aureus musculoskeletal infections in children. *Pediatr Infect Dis J* **23**(8): 701-6.

Mitchell PD, Hunt DM, Lyall H, Nolan M & Tudor-Williams G (2007) Panton-Valentine leukocidin-secreting Staphylococcus aureus causing severe musculoskeletal sepsis in children. A new threat. *J Bone Joint Surg Br* **89**(9): 1239-42.

Obando I, Croche B, Madrid D & Neth O (2011) [Deep vein thrombosis and septic pulmonary emboli associated with acute paediatric osteomyelitis due to methicillin-sensitive Staphylococcus aureus producing Panton-Valentine leukocidin]. *Enferm Infecc Microbiol Clin* **29**(7): 550-1.

Rafai M, Abouelalaa K, Skhsoukh Y, Balkhi H, Belyamani L & Dimou M (2013) Evolution of a fatal septic arthritis caused by a Panton-Valentine leukocidin (PVL)-producing Staphylococcus aureus strain. *Joint Bone Spine* **80**(5): 525-7.

Sdougkos G, Chini V, Papanastasiou DA, Christodoulou G, Tagaris G, Dimitracopoulos G & Spiliopoulou I (2007) Methicillin-resistant Staphylococcus aureus producing Panton-Valentine leukocidin as a cause of acute osteomyelitis in children. *Clin Microbiol Infect* **13**(6): 651-4.

Shallcross LJ, Fragaszy E, Johnson AM & Hayward AC (2013) The role of the Panton-Valentine leucocidin toxin in staphylococcal disease: a systematic review and meta-analysis. *Lancet Infect Dis* **13**(1): 43-54.

Smith IM, Austin OM & Batchelor AG (2006) The treatment of chronic osteomyelitis: a 10 year audit. *J Plast Reconstr Aesthet Surg* **59**(1): 11-5.

Szmigielski S, Prevost G, Monteil H, Colin DA & Jeljaszewicz J (1999) Leukocidal toxins of staphylococci. *Zentralbl Bakteriol* **289**(2): 185-201.

Tong SY, Lilliebridge RA, Bishop EJ, Cheng AC, Holt DC, McDonald MI, Giffard PM, Currie BJ & Boutlis CS (2010) Clinical correlates of Panton-Valentine leukocidin (PVL), PVL isoforms, and clonal complex in the Staphylococcus aureus population of Northern Australia. *J Infect Dis* **202**(5): 760-9.

Vander Have KL, Karmazyn B, Verma M, Caird MS, Hensinger RN, Farley FA & Lubicky JP (2009) Community-associated methicillin-resistant Staphylococcus aureus in acute musculoskeletal infection in children: a game changer. *J Pediatr Orthop* **29**(8): 927-31.