Long-term outcome of Cavalier King Charles spaniel dogs with clinical signs associated with Chiari-like malformation and syringomyelia


The disease complex Chiari-like malformation (CM) and syringomyelia (SM) has been associated with the development of neuropathic pain (NeP), and commonly affects Cavalier King Charles spaniels (CKCS). This prospective cohort study followed 48 CKCSs with CM and/or SM and clinical signs suggestive of NeP for a period of 39 (±14.3) months from diagnosis. At the end of the study, 36 dogs were still alive; five dogs died of an unrelated or unknown cause, and seven were euthanased due to severe clinical signs suggestive of NeP. During the follow-up period, the clinical signs of scratching, facial rubbing behaviour, vocalisation and exercise ability were evaluated. Nine out of 48 dogs stopped scratching (P < 0.001), but there was no statistically significant change in the number of dogs exhibiting exercise intolerance, vocalisation or facial rubbing behaviour. The overall severity of clinical signs based on a visual analogue scale (VAS) (0 mm: no clinical signs 100 mm: severe clinical signs) increased (from median 75 mm (interquartile ranges (IQR) 68–84) to 84 mm (IQR 71.5–91), P < 0.001). A quarter of the dogs were static or improved. In general, the majority of the owners felt that the quality of life of their dogs was acceptable. Medical treatments received were gabapentin or pregabalin and/or intermittently, carprofen. The owner’s perception of their animal’s progress, and progress based on VAS, had strong positive correlation (Spearman’s rank correlation (rs) 0.74, P < 0.001). Overall, this study suggests that clinical signs suggestive of NeP progress in three-quarters of CKCSs with CM and/or SM.

Introduction

Chiari-like malformation (CM) and syringomyelia (SM) is an enfeebling disease complex most prevalent in Cavalier King Charles spaniels (CKCS) (Rusbridge and others 2000). CM refers to the apparent mismatch in volume between the caudal brain structures and the caudal skull (Carrera and others 2009, Cross and others 2009, Driver and others 2010), and is associated with herniation of the cerebellum through the foramen magnum (Rusbridge and others 2000, Lu and others 2003). SM refers to accumulation of fluid within the parenchyma of the spinal cord, and is thought to result from CM and resultant changes in the dynamics of cerebrospinal fluid (CSF) flow through the foramen magnum and the cranial part of the cervical spinal cord (Pirnia and others 2000, Iskandar and others 2004, March and others 2005, Cerda-Gonzalez and others 2006, Rusbridge and others 2006, Cerda-Gonzalez and others 2009).

The estimated prevalence of CM in this breed varies from 92 per cent (Cerda-Gonzalez and others 2009) to 100 per cent (Couturier and others 2008, Carrera and others 2009). SM affects almost half of asymptomatic (as perceived by their owners) young CKCS, which increases up to 70 per cent at the age of six years (Couturier and others 2000, Parker and others 2011). The prevalence of CKCS with SM that suffer clinical signs suggestive of NeP is unknown.

Clinical signs typically associated with CM and/or SM include cervical scoliosis, thoracic limb ataxia, thoracic limb paresis and signs suggestive of neuropathic pain (NeP) (Rusbridge and others 2006). NeP most often manifests as allodynia (pain arising from a non-noxious stimulus, i.e. gentle palpation) or dysesthesia (spontaneous or evoked unpleasant sensation which manifests as phantom scratching, facial/ear rubbing) (Rusbridge and Jeffery 2008). One frequent sign of NeP attributed to dysesthesia and/or allodynia is phantom scratching. Phantom scratching is characterised by a pelvic limb scratching action to the shoulder and neck area, often without making skin contact, and typically on one side only. Unlike scratching associated with skin disease, dogs will often scratch whilst walking (Rusbridge and Jeffery 2008). It is not yet fully understood how CM/SM causes
NeP. However, histopathological studies of SM in CKCS have found that dogs which had expressed signs of NeP suffered an asymmetrical syrinx with profound alteration of the structure of the dorsal horn laminae, and had reduced expression of the pain-related neuropeptides substance P and calcitonin gene-related peptide (Hu and others 2012a). Glial and fibrous proliferation were also associated with expression of clinical signs (Hu and others 2012b). Chiari-I malformation/SM causes clinical signs of NeP in up to 90 per cent of human beings with this disorder, and up to 95 per cent of affected dogs (Todor and others 2000, Rusbridge and others 2007). NeP has an important impact on the affected person’s quality of life (QOL) and neurobehaviour (Gustorff and others 2008), and a recent study in dogs (Rutherford and others 2010) confirmed an association between the degree of NeP and fear/anxiety-related behavioural changes.

Medical and surgical treatment options exist for dogs with CM/SM. Medical management includes the use of NSAIDs, drugs that reduce CSF production (omeprazole, cimetidine), corticosteroids and antiepileptic drugs that have analgesic properties (gabapentin, pregabalin). However, there is no scientific evidence to prove the efficacy of these drugs in the management of NeP associated with CM/SM in dogs (Rusbridge and others 2006, Rusbridge and Jeffery 2007, Wölfte and Poma 2010). Surgical management (craniocervical decompression) is frequently performed in people with Chiari-I malformation, with and without SM, to alleviate clinical signs (Tubbs and others 2003). Following surgery, 60 per cent of dogs improved, but there was no resolution of the syringes, and nearly all dogs continued to exhibit clinical signs suggestive of NeP postoperatively (Vermeersch and others 2004, Dewey and others 2005, 2007, Rusbridge 2007). Additionally, 25–47 per cent of the operated dogs showed recurrence or deterioration of the clinical signs within 0.2–5 years after surgery (Dewey and others 2005, 2007, Rusbridge 2007).

To the authors’ knowledge there are currently no data regarding the long-term outcome of non-surgically managed dogs with clinical signs suggestive of NeP secondary to SM. This prospective cohort study follows 48 CKCS dogs with clinical signs suggestive of NeP due to CM and/or SM for a period of 39 (±14.3) months.

Materials and methods

Study design

A prospective cohort study was performed following 48 CKCS dogs with CM and/or SM disease complex for a mean period of 39 (±14.3) months from treatment termination.

Animals

CKCSs between the ages of one and 13 years (median 46 months), and bodyweight of 4–13 kg (median 9.5 kg), were recruited from the general population in the UK by advertising through the veterinary press and national CKCS health societies, into a two-week trial of a novel neuropathic pain medication, which was performed under the Animals (Scientific Procedures) Act 1986, and was approved by the institution’s ethics committee. After the end of the drug trial, dogs were followed prospectively for a mean period of 39 (±14.3 SD), 4–107 months for this study.

Dogs with other medical or neurological conditions that could have influenced the preceding pharmacological study were excluded, such as brain or spinal cord diseases, other than CM/SM; CKCS which had undergone craniocervical decompression; those with evidence of inflammation in the external ear canal (erythema, discharge, lichenification); with a history and clinical signs of skin disease, seizures at the time of diagnosis; or with a systolic heart murmur of greater than grade II/VI. Thus, owners were questioned about general health status, exercise intolerance and clinical signs suggestive of pruritus and pain. Recruitment and inclusion criteria were: CKCS with clinical signs suggestive of NeP (such as spinal hyperesthesia on palpation, facial rubbing, vocalisation and/or phantom scratching) (Rusbridge and others 2006, Rusbridge and Jeffery 2007) which scored 50 or more on the visual analogue scale (VAS), underwent MRI of the brain and spinal cord (Driver and others 2010, Loderstedt and others 2011), and were subsequently diagnosed with CM and/or SM by a board-certified neurologist. CM was defined as evidence of cerebellar herniation or indentation by the supraoccipital bone (Lu and others 2003), and a syrinx was defined as a fluid-containing cavity within the spinal cord parenchyma with a transverse diameter of greater than or equal to 2 mm (Driver and others 2010).

Assessment of clinical signs

A questionnaire was used to assess the following factors in a face-to-face interview at the initial visit, and then by telephone for the follow up: name of the animal and owner, sex of the animal and neutering status, date of birth and death, if applicable, whether it was euthanasia or natural death, cause of death, general health status, history of brain or spinal cord diseases other than CM/SM, if the dog had evidence of inflammation in the external ear canal, history and clinical signs of skin disease, seizures, exercise intolerance, whether there was another MR scan performed following their initial visit, if the animal underwent craniocervical decompression, if the animal had been diagnosed with a heart murmur, and what grade it was. The owners were also asked to confirm the presence of the following signs: phantom scratching of shoulder and/or neck, facial and ear rubbing, vocalisation and spinal pain. At the initial consult, all dogs were examined by a board-certified neurologist, and had unremarkable complete blood cell count and serum biochemistry profile. A VAS was used by the authors to assess the frequency and intensity of clinical signs suggestive of NeP. A 100 mm line ranging from 0 mm (asymptomatic dogs exhibiting normal exercise ability, no scratching, no facial rubbing and no vocalisation) to 100 mm (dogs with severely compromised exercise activity, scratching more than five times a day and vocalising more than five times a week) was used for the VAS assessment by intersecting the line with a second perpendicular line drawn by the observer, based on the subjective severity of the signs reported. To ensure consistency as much as possible between scorers, two of the authors, a responsible for the initial scoring underwent training, which involved independent scoring of clinical signs until their score varied less than three per cent. The follow-up information gathered by calling the owners was cross-referenced to the history from the referring veterinarian. The owners were asked what medication the animal received, and what was their perception of the progress of their animal’s condition (worse, unchanged, better) and QOL. The follow-up information was subsequently given a second score (VAS) by one of the authors. The author who assessed the VAS at the follow up was blinded to the initial score. In addition, the volume of the caudal cranial fossa, the parenchyma within the caudal cranial fossa, and the sizes of the ventricles and syringes were measured at the time of diagnosis using previously described methodology (Driver and others 2010) and compared with the progression of the assessed clinical signs.

Statistical analysis

Statistical analysis was performed with a commercial software package (Prism 5 for Mac, Graphpad Software 2007). Paired nominal categorical data were compared using the McNemar’s test. All quantitative data were assessed for normality of distribution with the D’Agostino and Pearson omnibus normality test and graphically. Means and SDs were calculated for normally distributed continuous data (means ±SD), and medians and interquartile ranges (IQR) were determined for non-parametric data (median IQR). A Wilcoxon’s signed rank test was used for statistical evaluation of paired, non-parametric data. The unpaired t-test (parametric data) or Mann-Whitney U test (non-parametric data) were used for comparing the morphometric values between dogs with and without deterioration of clinical signs assessed by VAS as appropriate. The association between owner’s perception of their animal’s progress (worse, unchanged, better) and progress based on VAS, was evaluated with Spearman’s rank correlation. A P value of 0.05 or below was considered significant.

Results

Sixty-one CKCS were initially considered for this study. Of these, eight were excluded due to the absence of clinical signs attributable to CM/SM (n=3), craniocervical decompression (n=1), grade III/VI systolic heart murmur (n=2), generalised pyoderma (n=1), and 4–107 months for this study.

---

1 K.E. Chandler
2 H.A. Volk
otus. Six dogs developed seizures during the study.

At the end of the study, 36 dogs (75 per cent, 95 per cent CI 62.75 per cent to 87.25 per cent) were still alive, four dogs died naturally at home with signs of suspected congestive heart failure (a postmortem examination was not performed), one dog was euthanased due to an aggressive ovarian tumour that had metastasised to other organs, and seven were euthanised due to severe signs of NeP. The mean age at diagnosis was 52.2 (±33.1) months, and the mean age at the end of the study, or death, was 92.3 (±30.5) months.

Overall, 59 dogs were treated medically with gabapentin (Neurontin, 10 mg /kg every 8–12 hours), pregabalin (Lyrica, 2–4 mg /kg every 8 hours) and/or intermittently carprofen (Rimadyl, 2 mg/kg every 24 hours) and nine dogs were not on any medication for the management of NeP. Nine out of 48 dogs stopped scratching (< P < 0.001), but there was no statistically significant change in the number of dogs exhibiting compromised exercise ability, vocalisation or facial rubbing behaviour. The median VAS of the clinical signs increased in the study population significantly (P < 0.001) from the initial median VAS of 75 mm (IQR 68–84 mm) to follow up VAS 84 mm (IQR 71.5–91 mm) (Fig 1). The severity of clinical signs based on VAS deteriorated in 36 (75 per cent, 95 per cent CI 62.75 per cent to 87.25 per cent) dogs. From these dogs, 51 (86 per cent, 95 per cent CI 76.18 per cent to 95.52 per cent) were treated with gabapentin or pregabalin, and carprofen. The remaining five dogs did not receive any treatment. A quarter of the dogs (95 per cent CI 12.75 to 37.25) were static (n=7, 14.5 per cent) or improved (n=5, 10.5 per cent).

The owner’s perception of their animal’s progress, and progress based on VAS, was strongly positively correlated (Spearman’s rank correlation (s) =0.74, P=0.001). All the owners of the dogs that were alive at the end of the study reported that their dog’s QOL was not severely compromised, and that it is responsible for the expression of gastrin-releasing peptide receptors (GRPR). These GRP receptors were found in a group of cavity collapse (Vaquero and others 2011). A possible explanation is that there was destruction of spinohalamic and lemniscal tracts by the syringes, so that pain could no longer be perceived (Hatem and others 2010). A further possibility is that the caudal cranial fossa and brain parenchyma adjust to compensate for the overcrowding of the caudal cranial fossa (Hamilton and others 2011).

Rusbridge (2007) followed up 15 CKCSs that underwent cranio-cervical decompression for 0.2–2.3 years after surgery, and despite an initial clinical improvement in 80 per cent of the dogs, 47 per cent deteriorated in a mean time of 1.3 years. Ten out of these 15 dogs had severe signs of NeP, and medical treatment was not successful. Despite the initial improvement though, all dogs continued to exhibit signs of NeP, and surgery did not change the size of the cervical syringes. Similar findings were described by Vermeersch and others (2004) and Dewey and others (2005, 2007). One cause of deterioration was attributed to scar tissue adhering to exposed neural tissue and preventing adequate CSF flow. Further studies that follow up cases for a longer period of time are needed to be able to compare the long-term outcome of conservative and surgical treatment, but it seems that cranio-cervical decompression may not have more favourable long-term outcomes in the management of neuropathic pain.

Nonetheless, the 48 dogs had CM only. Traditionally, clinical signs suggestive of NeP in CKCS have been associated with CM and SM, but from our study, we found that CM only may contribute to these signs, too. The pathophysiology of these clinical signs in dogs with CM is not well understood, but the overcrowding of the foramen magnum might be applying pressure onto brainstem nuclei causing signs suggestive of NeP (Rusbridge and Jeffery 2008). Unfortunately, there is no evidence for this theory, but in the human literature, there are several reports associating Chiari malformation with trigeminal neuralgia that resolves after cranio-cervical decompression or placement of vertebropletenoneal shunt (Gnanalingham and others 2005, Vinc and others 2010).

Interestingly, there was a significant improvement in scratching (nine dogs stopped scratching), whereas, the VAS got significantly worse. New research findings in the area of pruritus may give an explanation for this. Pruritus, traditionally, has been associated with a submodality or subquality of pain (Sun and others 2009). Advances in this area have elucidated differences between pruritus and pain, but have also obscured the distinction between them. Pruritus and pain appear to be independent sensations because nociceptive and pruriceptive stimuli each elicit unique behavioural responses (Davidson and others 2010). Sun and Chen (2007) reported that they have identified the first gene in the spinal cord of mice, linked with pruritus, and that it is responsible for the expression of gastrin-releasing peptide receptors (GRPR). These GRP receptors were found in a group.

FIG 1: Scatter plots showing the distribution of Visual Analogue Scale (mm) in Cavalier King Charles spaniel dogs with clinical signs at the beginning of the study (initial) and 39±14.3 months later (follow up) (bar represents median; Wilcoxon’s signed rank test, *represents P < 0.001)

Discussion
To the authors’ knowledge, this is the first report of clinical signs suggestive of NeP associated with CM/SM being progressive in the majority of dogs when treated non-surgically. In this study, morphometric values did not seem to play a significant role in the progression or improvement of the clinical signs. Three quarters of the dogs displayed progression of clinical signs, whereas, one quarter remained static or improved. Despite the deterioration of the clinical signs in the majority of these dogs, 75 per cent were still alive 39±14.3 months later, with an acceptable QOL for the owners. All the owners of these dogs indicated that if their dogs’ QOL was severely compromised, then they would opt for euthanasia. This study shows that non-surgical management of this condition can be an acceptable option considering that there is no evidence for better management in dogs.

The clinical course of the clinical signs seen in our study is similar to what has been reported in human beings with unoperated SM associated with type I Chiari malformation, and/or underdevelopment of posterior fossa (Bogdanov and Mendelevich 2002). It has been suggested that the natural history of symptomatic SM associated with type I Chiari malformation, and/or signs of posterior fossa underdevelopment, is characterised by an initial relatively rapid clinical progression accompanied with distended cavities. If surgery is not performed, then a state of syrinx equilibrium may be reached, that is, no further expansion of the syrinx, although this may be associated with irreversible spinal cord damage. Eventually, in some cases, there may be MRI signs of cavity collapse (Vaqueiro and others 2011). A possible explanation is that there was destruction of spinohalamic and lemniscal tracts by the syringes, so that pain could no longer be perceived (Hatem and others 2010). A further possibility is that the caudal cranial fossa and brain parenchyma adjust to compensate for the overcrowding of the caudal cranial fossa (Hamilton and others 2011).

Rusbridge (2007) followed up 15 CKCSs that underwent cranio-cervical decompression for 0.2–2.3 years after surgery, and despite an initial clinical improvement in 80 per cent of the dogs, 47 per cent deteriorated in a mean time of 1.3 years. Ten out of these 15 dogs had severe signs of NeP, and medical treatment was not successful. Despite the initial improvement though, all dogs continued to exhibit signs of NeP, and surgery did not change the size of the cervical syringes. Similar findings were described by Vermeersch and others (2004) and Dewey and others (2005, 2007). One cause of deterioration was attributed to scar tissue adhering to exposed neural tissue and preventing adequate CSF flow. Further studies that follow up cases for a longer period of time are needed to be able to compare the long-term outcome of conservative and surgical treatment, but it seems that cranio-cervical decompression may not have more favourable long-term outcomes in the management of neuropathic pain.

Nonetheless, the 48 dogs had CM only. Traditionally, clinical signs suggestive of NeP in CKCS have been associated with CM and SM, but from our study, we found that CM only may contribute to these signs, too. The pathophysiology of these clinical signs in dogs with CM is not well understood, but the overcrowding of the foramen magnum might be applying pressure onto brainstem nuclei causing signs suggestive of NeP (Rusbridge and Jeffery 2008). Unfortunately, there is no evidence for this theory, but in the human literature, there are several reports associating Chiari malformation with trigeminal neuralgia that resolves after cranio-cervical decompression or placement of vertebropletenoneal shunt (Gnanalingham and others 2005, Vinc and others 2010).

Interestingly, there was a significant improvement in scratching (nine dogs stopped scratching), whereas, the VAS got significantly worse. New research findings in the area of pruritus may give an explanation for this. Pruritus, traditionally, has been associated with a submodality or subquality of pain (Sun and others 2009). Advances in this area have elucidated differences between pruritus and pain, but have also obscured the distinction between them. Pruritus and pain appear to be independent sensations because nociceptive and pruriceptive stimuli each elicit unique behavioural responses (Davidson and others 2010). Sun and Chen (2007) reported that they have identified the first gene in the spinal cord of mice, linked with pruritus, and that it is responsible for the expression of gastrin-releasing peptide receptors (GRPR). These GRP receptors were found in a group.
of spinal cord cells called lamina-I neurones that relay both itch and pain sensation to the brain. However, Sun and others (2009) believe that there is a specific subpopulation of GRPR neurones, located in the superficial dural horn within the lamina-I, that are specific only for the pruriticeptive sensation (labelled-line hypothesis). Destruction of the described neurones in mice and stimulation afterwards with various pruriticeptive agents showed up to an 85% reduction in pruriticeptive sensation compared with the controlled group, but nociception and motor function appeared to be unaffected (Sun and others 2009). Many attempts to localise the pathway of these GRPR neurones all the way up to the thalamus (where they finally project) failed, but it is still believed that the pruriticeptive pathway is different to the nociceptive pathway in the spinothalamic tract neurones that project to the posterior and ventral posterior region of the thalamus (Sun and others 2009). Further research is needed to elucidate the difference between these two pathways.

Another interesting finding of this study is that despite the fact that history of seizures at the time of diagnosis was an exclusion criterion, six dogs (12.2 per cent) developed seizures in the investigated period of time. It was suggested that seizures may be related to ventriculomegaly secondary to overcrowding of the cranial fossa (Rushbridge and others 2006), however, a recent study could not show a relationship between ventriculomegaly and seizures (Driver and others 2012). This finding is more likely related to idiopathic epilepsy which is common in this breed (Rushbridge and Knowler 2004) or other unknown pathologies. In human beings, epilepsy, in conjunction with type I Chiari malformation, is occasionally reported. Two subtypes are described: the first as an incidental finding in the diagnostic work-up of patients with idiopathic epilepsies, and the second where both type I Chiari malformation and epilepsy occur as part of a more widespread developmental disorder (Granata and Valentini 2011).

In three out of eight female entire bitches, the owners reported periodical aggravation of the CM/SM-related clinical signs during oestrous. Hubser and others (2010) reported that oestrogen (17b-estradiol) administration reduces experimentally induced allodynia in rats, but there is no literature to support that oestrogens can deteriorate the signs of NeP. To the authors’ knowledge, there is no involvement of oestrogens in the pathophysiology of NeP, and this may be an interesting finding that requires further investigation. Perhaps the stress associated with the oestrous can aggravate the perception of NeP, in a similar way that mood affects the pain perception in human beings (Gustoff and others 2008).

There are limitations to this study, considering the selection criteria. Only with 5 or more underdiagnosed MRI had were included in the study. However, we do not feel that selecting for dogs with prominent clinical signs is a significant limitation of the study as they reflect the general population of dogs that are presented to a neurologist for investigation of this condition. There is possible bias from the owners and the veterinary surgeons when assessing the clinical signs. Also, one of the most important problems is that pain is a subjective variable and may have been inappropriately assessed considering that the dogs cannot verbally communicate their level of discomfort. Moreover, we can only assume that CMS/M is the cause of the described clinical signs and that these signs are suggestive of neuropathic pain. A follow-up MRI was not performed, so we cannot exclude the possibility of development of other spinal diseases in the study period that may affect the progression of the clinical signs. The VAT itself, as with other scoring systems, is subject to bias, and its reproducibility can be questioned; however, the scorers in this study were trained to be as consistent as possible in their observations. The VAT is a recognised tool for measuring subjective phenomena, such as anxiety, pain, QOL (Bond and others 1995, Grunberg and others 1996, Cox and others 2005), and it has been used extensively in people, and increasingly in veterinary literature. It seems to be reliable (Boonstra and others 2008, Hijlum-Bjorkman and others 2011), more responsive (Scrinshaw and others 2001), and easy to use, compared with other pain-scoring systems. When reviewing the length of this cohort study, euthanasia was a factor. The ability for human owners to eliminate their animals’ suffering through euthanasia is not a shared ethical dilemma with human sufferers of CM/SM complex. For each owner, this will be an individual decision, and based on the bond they have with their pets, and also the potential economic decisions. Finally, it is important to mention that this study is documenting the progression of a specific set of clinical signs in a group of dogs with CM and/or SM, and not the progression of the disease itself. Radiological progression of the disease has not yet been reported. Fifteen per cent (95 per cent CI 4.9 to 25.1) of the study population was euthanased at the request of the owner due to the severity of the clinical signs. However, in the remaining surviving dogs, despite the progression of the clinical signs, the majority of the owners felt that QOL of their dogs was acceptable.

Acknowledgements

The authors would like to acknowledge the owners of the patients who participated in this study, and the veterinarians who referred them to us. The authors would like to thank the various CKCS health clubs for their continuous support for the investigation of these conditions. Finally, the authors also would like to thank the Clinical Investigation Centre of the Royal Veterinary College and its research office for assessing the manuscript according to the Royal Veterinary College’s code of good research practice (authorisation number: VCS_00225).

References


RUSBRIDGE, C. & KNOWLER, S. F. (2004) Inheritance of occipital bone hypoplasia (Chiari type 1 malformation) in Cavalier King Charles spaniels. *Journal of Veterinary Internal Medicine* 18, 673–676


Long-term outcome of Cavalier King Charles spaniel dogs with clinical signs associated with Chiari-like malformation and syringomyelia


Veterinary Record published online October 25, 2012
doi: 10.1136/vr.100449

Updated information and services can be found at:
http://veterinaryrecord.bmj.com/content/early/2012/10/24/vr.100449.full.html

These include:

References
This article cites 42 articles, 4 of which can be accessed free at:
http://veterinaryrecord.bmj.com/content/early/2012/10/24/vr.100449.full.html#ref-list-1

P<P

Published online October 25, 2012 in advance of the print journal.

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

Advance online articles have been peer reviewed, accepted for publication, edited and typeset, but have not yet appeared in the paper journal. Advance online articles are citable and establish publication priority; they are indexed by PubMed from initial publication. Citations to Advance online articles must include the digital object identifier (DOIs) and date of initial publication.

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/