Canine Atlantoaxial Ventral Stabilization: Computed Tomography Analysis of Optimal Safe Implantation Corridors and Comparison of the Technical Outcome and Biomechanical Properties of 3 Surgical Techniques

by

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CANINE ATLANTOAXIAL VENTRAL STABILIZATION: COMPUTED TOMOGRAPHY ANALYSIS OF OPTIMAL SAFE IMPLANTATION CORRIDORS AND COMPARISON OF THE TECHNICAL OUTCOME AND BIOMECHANICAL PROPERTIES OF 3 SURGICAL TECHNIQUES

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Canine atlantoaxial instability is a severely debilitating condition most commonly affecting Toy breed dogs that can result in sudden cardio-respiratory arrest. Surgical correction of atlantoaxial subluxation requires positioning of implants into the atlas and axis which is often perceived as a hazardous procedure and has been associated with unacceptably high perioperative mortality rates. This thesis investigates the most commonly reported ventral techniques of atlantoaxial stabilization attempting to improve currently available technical descriptions.

The first part was aimed at developing a method of 3D analysis of vertebral bone corridors in order to generate precise definitions of optimal implant placement that could be used intraoperatively. We developed and validated a semi-automated method using free open-source version of OsiriX™ DICOM viewer and Microsoft® Excel software programs. This method was subsequently applied to a population of 27 dogs to generate objective definitions of atlantoaxial optimal safe implantation corridors.

In the second part, we used these definitions to position implants in a population of 21 Beagle cadavers simulating intraoperative conditions of atlantoaxial stabilization. A 3D drill guide prototype was designed to allow positioning of the implants according to
the previously generated definitions. Three commonly reported techniques of ventral atlantoaxial stabilization and 5 different implant sites were statistically compared. This study provided insights on technical limitations of the different methods of stabilization as well as biomechanical data that should be considered when performing these surgeries in clinical cases.
Acknowledgments

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Declaration of work performed

I declare that, with the exception of the items below, all work in this thesis was performed by me.

Computed tomography scans were performed with the help of Carolyn Bennett and Sheila Currie. Image interpretation and angle measurements obtained to validate our method of optimal safe implantation corridor analysis were performed in collaboration with Dr. Alex zur Linden. General anesthesia was supervised by the Ontario Veterinary College Health Sciences Centre Anesthesia Service. Surgical stabilization procedures were carried out in collaboration with Dr. Noel Moens with the assistance of Amanda Hathway and Nicole Kudo.

The design of our jig for biomechanical testing was developed in collaboration with Dr. John Runciman. The machining of the jig was done by Ken Graham at the University of Guelph School of Engineering. The manufacturing of our 3D drill guide prototype was performed by Mario Paroutis at the University of Guelph Physics Department. Anatomical preparation of the vertebrae following biomechanical testing was performed in collaboration with Roman Poterski from of the department of Biomedical Sciences.

Statistical analysis was performed by Gabrielle Monteith.
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List of abbreviations

2D: Bidimensional
3D: Tridimensional
AA: Atlantoaxial
AAI: Atlantoaxial instability
ANOVA: Analysis of variance
C1: Atlas
C2: Axis
Cd: Caudal
Cr: Cranial
CritSafA: Critical safety angles
CritTecA: Critical technical angle
CT: Computed tomography
D or Dors: Dorsal
DICOM: Digital Imaging and Communication in Medicine
EZ: Elastic zone
L: Left
Lat: Lateral
MI: Multi-implant
MI5: Multi-implant polymethylmethacrylate cemented construct using 5 screws
MI6: Multi-implant polymethylmethacrylate cemented construct using 6 screws
MPR: Multi-planar reconstruction
MRI: Magnetic resonance imaging
N-Beagle: Non-affected Beagle
N-Toy: Non-affected Toy breed
NZ: Neutral zone
OSIC(s): Optimal safe implantation corridor(s)
OVC HSC: Ontario Veterinary College Health Science Centre
PFZ: Pain free zone
PMMA: Polymethylmethacrylate
ProjA: Projected angle
R: Right
ROI: Region of interest
ROM: Range of motion
SafA: Safety angle
Sag: Sagittal
SCI: spinal cord injury
SD: Standard deviation
Tr: Transverse
TrA: Transarticular
TSF: Transarticular screw fixation
V or Vent: Ventral
VB: Vertebral body
VC: Vertebral canal
VR: Volume rendering

Mathematical notations:
“.”: Multiplication
“×”: Cross product of vectors
“•”: Dot product of vectors
\( \vec{X} \): vector X
\( \|X\| \): norm of vector X
\( \hat{X} \): unit vector X (normalized)
List of trademarks and registered products

Apple® computer Mac mini (Apple Inc., Cupertino, CA, USA)

Bluehill®3 (Illinois Tool Works Inc., Instron Co., Norwood, MA, USA)

Cage Assembly Rod Ø6 mm (Thorlabs Inc., Newton, NJ, USA).

CorelDRAW® Graphic Suite version X7 (Corel Corp., Ottawa, ON, Canada)

Dremel® (Robert Bosch Tool Corp., Mount Prospect, IL, USA)

Instron® Model 5965 (Illinois Tool Works Inc., Instron Co., Norwood, MA, USA)

Microsoft® Excel software version 2011 (Microsoft Corp., Redmond, WA, USA)

OsiriX™ versions 5.8-6.0 (Pixmeo SARL, Bernex, Switzerland)

Palacos® R (Zimmer, Inc., Warsaw, IN, USA)


Vacu-Positioner Pad® (Shor-Line, Kansas City, KS, USA)
CHAPTER I - GENERAL LITERATURE REVIEW

I. Introduction

The first case of canine atlantoaxial (AA) instability was reported by Downey in 1967. In his publication, Downey described a case of odontoid process agenesis in a miniature Poodle that suffered from acute tetraplegia following a fall. Agenesis of the odontoid process was diagnosed by radiography and the dog responded well to conservative management. One month later, Geary et al. (1967) described the first technique for surgical stabilization of the AA joint in a case series. Those publications were subsequently followed by multiple case reports and case series outlining the clinical difficulties associated with this condition and resulted in the description of numerous variations of the surgical techniques in an attempt to improve the overall outcome (LeCouteur, McKeown, et al. 1980, Sorjonen & Shires 1981, Kishigami 1984, Schulz, Waldron, et al. 1997). To this day, AA instability remains a significant challenge in veterinary neurosurgery and new methods of stabilization continue to be explored (Dickomeit, Alves, et al. 2011, Aikawa, Shibata, et al. 2013, Sanchez-Masian, Lujan-Feliu-Pascual, et al. 2014, Stalin, Gutierrez-Quintana, et al. 2015).

AA instability has been reported in multiple species including dogs, cats, horses and humans (Thomson & Read 1996, Licka & Edinger 2000, Arvin, Fournier-Gosselin, et al. 2010, El-Khoury, Mourao, et al. 2014). In veterinary medicine this disease is most commonly observed in dogs and therefore this species will remain our focus.

The terminology for this condition varies vastly in the literature. The most commonly used terms in the recent literature are atlantoaxial subluxation and atlantoaxial instability.
The latter will be used preferentially in this text since we consider it provides a better description and understanding of the underlying pathophysiology. AA instability represents only one entity within a larger group known as craniocervical junction anomalies. Those anomalies include AA instability, atlanto-occipital overlapping, caudal occipital malformations, occipitoatlantoaxial malformations and craniocervical junction dorsal fibrous bands (Cerda-Gonzalez & Dewey 2010, Westworth & Sturges 2010, Dewey, Marino, et al. 2013).
II. Anatomy and physiology of the atlantoaxial joint

Anatomy of the atlantoaxial joint

The first and second cervical vertebrae (atlas and axis) have a highly specialized conformation which provides the head its wide multi-directional range of motion (Watson, Evans, et al. 1986b, Evans & de Lahunta 2012). The atlas (C1) has a markedly enlarged vertebral foramen (foramen vertebrale) due to a reduced size of the vertebral body that is more appropriately described as a ventral arch (arcus ventralis). The dorsal surface of the ventral arch forms a concave surface (fovea dentis) articulating with the odontoid process (dens) of the axis (C2). In contrast the pedicles are enlarged to form thick lateral masses (massa lateralis) which support the concave cranial and caudal articular surfaces (foveae). These foveae are paired and positioned laterally. The cranial foveae (foveae articularis cranialis) articulate with the occipital condyles whereas the caudal foveae (foveae articularis caudalis) articulate with the cranial articular surfaces of the axis. Other major atypical features of C1 include the absence of a spinous process on the dorsal arch (arcus dorsalis) and its wide lateral processes named atlas wings (alae atlantis). The wings form a shallow cavity ventrally named fossae (fossae atlantis).

Important anatomical landmarks of C1 include the paired alar notches (incisurae alaris), the ventral tubercle (tuberculum ventrale) and, to a lesser extent, the dorsal tubercle (tuberculum dorsale).

The axis is also a greatly modified vertebra (Evans & de Lahunta 2012). One of the most striking features of C2 is its prominent spinous process (processus spinosus) which extends cranially over the dorsal arch of C1 and caudally over the lamina of the third cervical vertebra. It is a thin structure cranially which broadens caudally to join the paired caudal articular processes (processus articularis caudalis). The cranial portion of the
vertebral body extends dorsally to the ventral arch of C1 forming a cranial tooth-like structure named odontoid process (dens), where its cranial extremity is termed the apex. The cranial articular surfaces of C2 are placed laterally on either side of the dens. The overall shape of C2 is elongated but otherwise, pedicles (pediculus arcus vertebrae), laminae (lamina arcus vertebrae) and transverse processes (processus transversus) can be recognized similarly to other cervical vertebrae. Important anatomical landmarks of C2 include its ventral median crest (crista ventralis) separating 2 deep fossae, the cranial and caudal extremities of the ventral crest that forms small eminences and the transverse processes.

The occipital bone (os occipitalis) is an important element of the craniocervical junction (Evans & de Lahunta 2012). The paired lateral parts (partes laterales) support the occipital condyles (condyli occipitales) which articulate with the cranial foveae of C1. This joint allows most of the ventrodorsal motion of the head in regard to the atlas. The basilar part (pars basilaris) forms the ventral border of the foramen magnum whereas the squamous or supraoccipital part (squama occipitalis) forms the dorsal limit of the foramen magnum. Multiple malformations of the occipital bone are described in the literature such as occipital condyle anomalies, atlantooccipital overlapping, occipital hypoplasia and Chiari-like malformations. These anomalies do not typically result in AA instability but can be associated with odontoid process or ligamentous anomalies and aggravate clinical signs of cervical myelopathy (Rylander & Robles 2007, Cerda-Gonzalez & Dewey 2010, Galban, Gilley, et al. 2010, Dewey, Marino, et al. 2013).

The craniocervical junction forms a complex synovial joint ventrally named the occipitoatlantoaxial joint providing articulation to the skull, atlas and axis together (Evans & de Lahunta 2012). Even though the AA joint (articulatio atlantoaxialis) can be considered as a separate functional unit, it cannot be anatomically differentiated from the
atlantooccipital joint (*articulatio atlantooccipitalis*) due to their close inter-relationships. A synovial joint cavity is formed by the fusion of the paired atlantooccipital joints, the single central joint articulating the dens and C1 ventral arch, and the paired AA joints (Watson, Delahunta, *et al*. 1986, Watson, Evans, *et al*. 1986a). A dorsal synovial bursa is located between the dens and the transverse ligament that remains separated from the joint (Evans & de Lahunta 2012).

The atlas and axis are supported by a synovial joint capsule (*capsula articularis*), paraspinal muscles and multiple ligaments. It is important to consider that most of these structures connect loosely C1 and C2 allowing the wide range of motions of C2 around its longitudinal axis. The odontoid process functions as the main pivot of the joint and therefore it is also the main site of tight ligamentous stabilization. It is maintained in its anatomical position by 4 main ligaments: the apical ligament (*lig. apicis dentis*), the alar ligaments (*ligg. alaria*) and the transverse ligament (*lig. transversum atlantis*). The apical ligament (*lig. apicis dentis*) connects the apex of the odontoid process to the ventral edge of the foramen magnum (Fig I.1). The paired alar ligaments (*ligg. alaria*) connect the dens to the medial surface of the occipital condyles, although a recent anatomical study suggested that part of the alar ligament also inserts on the dorsal surface of the ventral arch of C1 (Kupczynska, Wieladek, *et al*. 2012). The transverse ligament of the atlas (*lig. transversum atlantis*) connects the right and left lateral masses and maintains the dens positioned against the ventral arch of C1 (Evans & de Lahunta 2012). In humans, the tectorial membrane (*membrana tectoria*) has been suggested to participate in maintaining the odontoid process against the C1 ventral arch. However, this structure extending the dorsal longitudinal ligament cranially is poorly developed in dogs and rarely described in the literature (Tubbs, Kelly, *et al*. 2007, Middleton, Hillmann, *et al*. 2012). Other fibrous structures involved in AA stability include the dorsal AA ligament (attaching the dorsal arch of the atlas to the spinous process of the axis), the synovial

**Fig I.1** Atlantoaxial osseous anatomy and main stabilizing structures.  

Multiple vital structures are located within or close to the AA joint. Surgically approaching the AA joint requires an extensive knowledge of the interrelationships existing between these vital structures and surrounding tissues. The most important vital structure is the spinal cord in the vertebral canal connecting through the foramen magnum to the medulla oblongata. The first pair of spinal nerves emerges from the
vertebral canal through the C1 dorsal arch at the level of the lateral vertebral foramen (*foramen vertebrae laterale*) (Evans & de Lahunta 2012). In some dogs, this foramen can be open cranially forming a notch in the dorsal arch (Richards & Watson 1991). The second pair of cervical spinal nerves leave the vertebral canal through an unusually large intervertebral foramen (*foramen intervertebrale*) formed by the caudal vertebral notch (*incisura vertebralis caudalis*) of C1 and cranial vertebral notch (*incisura vertebralis cranialis*) of C2. The vertebral arteries (*aa. occipitalis et vertebralis*) providing blood supply to the AA region run through the transverse foramen (*foramen transversarium*) of each pedicle from C6 to C2 vertebrae (Sharp & Wheeler 2005, Evans & de Lahunta 2012). At each segment, 3 branches separate from the vertebral artery: the spinal branches supplying the spinal cord and the dorsal and ventral muscular branches. Once the vertebral artery reaches C1, it branches dorsally against the dorsal surface of the atlas wing and ventrally through the alar foramen (*foramen alare*). Both branches anastomose with a branch of the occipital artery (artery also contributing to the blood supply of the AA region) which then travels with the first spinal nerve through the lateral vertebral foramen. In some dogs, an intraosseous canal crossing from the atlantal fossa to the vertebral canal through the lateral masses is described (Evans & de Lahunta 2012). This canal likely carries a small branch of the vertebral artery, although this has not been thoroughly studied. The intervertebral veins (*vv. intervertebrales*) arise from the ventral internal vertebral plexus (*plexus vertebralis internus ventralis*) and then joins the vertebral veins (*v. vertebralis*) which then follow the vertebral arteries through the alar and transverse foramen (Sharp & Wheeler 2005, Evans & de Lahunta 2012).
Embryological and postnatal development of the atlas and axis

In the early embryo, the longitudinal support of the body is provided by the notochord. As further development occurs, the notochord is replaced by segmented sclerotomes which will later form the vertebrae (Watson, Evans, et al. 1986b, Menezes 2008a, Evans & de Lahunta 2012). The notochord will only persist within the intervertebral discs. Early chondrification occurs within the sclerotomes as well as organization of the neural arches (dorsally) and the body and transverse/costal processes (ventrolaterally) to form structures resembling vertebrae by day 25 of gestation. In most vertebrae, ossification subsequently occurs from 3 primary centers (1 centrum and 2 neural arches). However, the atlas and axis embryological development differs greatly from the rest of the spine due to the fusion of several sclerotomes. The craniocervical junction originates from 3 sclerotomes which are the 4th occipital (also named pro-atlas), and the first 2 spinal sclerotomes. The axis body is formed from the fusion of the pro-atlas centrum, C1 centrum, C2 intercentrum and C2 centrum. The atlas body is formed from the C1 intercentrum. Initial ossification of the body of C1 and C2 dens occurs during fetal life between 42-46 days. The C2 intercentrum is partially ossified at birth, but complete fusion of the axis ossified elements occurs at approximately 4-5 months post-partum (Watson, Evans, et al. 1986b).

Biomechanics of the normal atlantoaxial joint

Modern spinal biomechanics subdivides the spine into multiple functional spinal units (or motion segments) each consisting of 2 vertebrae and their connecting structures (Panjabi 1992a, Panjabi 1992b, Hettlich, Allen, et al. 2013, Izzo, Guarnieri, et al. 2013a). A motion segment is capable of all 3 rotation motions (flexion/extension, lateral bending and axial rotation) and 3 translation motions (lateral translation, ventrodorsal translation
and compression/tension). These motions are also known as degrees of freedom.

Physiological spinal stability is defined by establishing the range of motion (ROM) of each motion unit in all 6 major degrees of freedom. The ROM is a vertebral displacement value defined as the relative motion between the neutral point and the point of maximal stiffness using the load/displacement curve of the motor unit (Fig I.2). The ROM of the spine is physiologically non-linear and biphasic and can therefore be subdivided into 2 major components named neutral zone (NZ) and elastic zone (EZ). The NZ corresponds to the initial motion away from the neutral position generated by minimal load due to physiological laxity of the supporting structures (capsules, ligaments and tendons). The EZ corresponds to the portion of the ROM generated by rapidly increasing loads due to tension forces generated by the supporting structures limiting spinal motions. The stiffness of the motor unit is the slope of the load/displacement curve and is a measure of the motor unit resistance to the applied motion. The determination of ROM, NZ, EZ and stiffness is considered an objective representation of the intervertebral laxity and stability. Determination of failure loads is also of interest when developing stabilization and joint replacement techniques.

**Fig I.2** Typical load/displacement curve of a spinal motion unit.

The range of motion (ROM) can be divided in 2 zones, the neutral zone (NZ) = slowly increasing loads and the elastic zone (EZ) = rapidly increasing loads.

There is currently limited data on the normal biomechanics of the craniocervical junction in dogs. Traditionally the occipitoatlantoaxial joint was viewed as a complex
synovial joint with 2 major separate components. The occipitoatlantal joint articulating
the cranial articular surfaces of C1 with the occipital condyles, was considered
responsible of ventral flexion and extension of the skull. Whereas the AA joint
articulating the caudal articular surfaces and ventral arch of C1 with the lateral cranial
articular surface and odontoid process of C2, was considered responsible for axial
rotation and lateral bending of both the skull and C1 (Evans & de Lahunta 2012).
However, this description is probably an oversimplified view of the craniocervical
junction. The complex anatomical interrelationships of the craniocervical motor units
suggest equally complex distribution of forces and motions.

A radiographic study of the canine cervical spine range of motions performed in
Beagles described that lateral bending occurred at both the occipitoatlantal (mean ROM
of 18°) and AA levels (not quantified due to positioning difficulties) (Morgan,
Miyabayashi, et al. 1986). Similarly, ventral flexion/extension was observed at the
occipitoatlantal joint (mean ROM of 86°) and AA joint. The latter ROM was quantified by
measuring the distance between the caudal margin of C1 dorsal arch and the cranial
margin of C2 spinous process on lateral views. They observed an overlap between
those 2 reference points of at least 0.40 ± 1.35mm in ventral flexion and up to 9.70 ±
1.40mm in extension. More recently, a biomechanical study using Beagle dogs as a
model for human atlanto-odontoid replacement described physiological rotational ROM
of the AA joint (Zang, Liu, et al. 2015). The authors reported ROM (mean ± SD) of 19.40
± 2.75° in flexion, 26.53 ± 3.77° in extension, 17.76 ± 1.05° in lateral bending, and 40.12
± 2.06° in axial rotation. They also reported NZ of 4.34 ± 0.32° in flexion/extension, 1.01
± 0.21° in lateral bending and 2.28 ± 0.18° in axial rotation. Lastly, they established
stiffness of the joint when submitted to 0.75Nm as 0.0444 ± 0.0062Nm/° in flexion,
0.0314 ± 0.0049Nm/° in extension, 0.0449 ± 0.0026Nm/° in lateral bending, and 0.0199
± 0.0011Nm/° in axial rotation.
As stated previously, the major stabilizing structures of the AA joint are associated to the odontoid process (i.e. transverse, apical and alar ligaments). This statement was mostly based on anatomical description (Watson, Delahunta, et al. 1986, Watson, Evans, et al. 1986a, Evans & de Lahunta 2012). However recent biomechanical studies have demonstrated increased ROM and NZ via odontectomy and/or ligament transections (Reber, Burki, et al. 2013, Zang, Liu, et al. 2015). One study demonstrated an increase in ROM and NZ compared to physiological values in Beagles that had their odontoid process resected as well as portion of the ventral arch of C1, transverse ligament and tectorial membrane (Zang, Liu, et al. 2015). Another study evaluated the effect of transecting the major AA ligaments on the ROM of the AA joint under shear load (ventrodorsal translation) (Reber, Burki, et al. 2013). The authors concluded that transecting the alar ligaments resulted in the most significant increase in ROM and therefore they represented the most significant stabilization structure against ventrodorsal shear forces. This study had several limitations, the most significant being that the ligaments were tested in the same order in all specimens. Therefore those results should be interpreted with caution. This finding is nevertheless interesting as it differs from what would have been expected in people where the transverse ligament is considered the main stabilizing structure of the odontoid process. The authors hypothesized that the horizontal position of the neck in dogs may increase the strain exerted on the ligamentous structure of the AA joint to support the head in comparison to humans. Further investigation will be necessary to determine if this finding is clinically relevant. Using basic biomechanics principles, it can be expected that major forces applied on the AA joint will differ greatly between humans and dogs (particularly in the standing position). In humans most of the weight of the head is directly transmitted to the vertebral column due to the bipedal standing position (roughly aligning the head’s center of gravity with the vertebral column axis). In dogs the weight of the head generates a
ventral bending moment on the cervical spine due to the forward position of its center of gravity. Therefore, in humans it can be anticipated that major forces applied on the cervical spine in a standing position will be compressive forces, whereas in dogs the cranially placed ventral bending moment will result in compressive forces ventrally, tension forces dorsally as well as dorsoventral shear forces (Fig I.3).

Fig I.3 Schematic representation of the main types of forces applied on the craniocervical junction. The weight of the head (in purple) generates a ventrally directed force at its centre of gravity. This results in a ventral bending moment applied on the cervical spine. Multiple resulting forces can be predicted including rotational forces (flexion, in green) and translational forces (ventrodorsal shear, in pink / ventral compression, in orange / dorsal tension, in blue). The atlantoaxial joint has been described as the zone of maximal strain due to caudal tension applied by the nuchal ligament and caudal muscles insertions.

Another important stabilization structure is the dorsal AA ligament connecting the dorsal arch of C1 to the spinous process of C2 (Fig I.1). Some authors have hypothesized that this ligament may provide enough stability to the AA join to maintain normal function or significantly limit the degree of instability (Stalin, Gutierrez-Quintana,
et al. 2015). This could explain why dogs suffering from agenesis of the odontoid process present with very chronic clinical signs and can remain asymptomatic for several years.
III. Pathogenesis and clinical presentation of atlantoaxial instability

Pathogenesis of atlantoaxial instability


In clinical cases, obvious radiographical evidence of subluxation is commonly reported although the severity of clinical signs does not always correlate with the degree
of vertebral displacement. This can be attributed in part to the reserve space around the spinal cord which is greater in the AA articulation than in other regions of the spine (Arvin, Fournier-Gosselin, et al. 2010). Some dogs may have some physiologic individual variations of the reserve space or congenital malformations (such as agenesis of C2 dens or C1 dorsal arch) which could result in greater tolerance to dorsal displacement of the axis.

The most commonly reported cause of canine AA instability is the malformation of the odontoid process. A systematic review reported that 24% of cases had an aplastic dens and 32% had a hypoplastic dens (Plessas & Volk 2014). Separation of the odontoid process without evidence of trauma has been known since the very early descriptions of the disease. However the origin of this separation remains for debate (Geary, Oliver, et al. 1967, Ladds, Guffy, et al. 1971). Some authors have speculated that a degenerative cause similar to Legg-Perthes disease could be involved (de Lahunta & Glass 2008). However, this would imply that normal ossification occurs prior to degeneration which is in contradiction with some reports where complete fusion of the dens is thought to have never occurred (Ladds, Guffy, et al. 1971). Whether some trauma is always involved in the separation of the dens or whether the congenital malformation results in the inability of the AA joint to support physiological head motions is also unclear. A similar debate exists in human medicine in a condition named os odontoideum (separated dens) where both congenital and acquired mechanisms have been advocated (Arvin, Fournier-Gosselin, et al. 2010).

Ligament dysplasia has been also reported in the literature (Watson & de Lahunta 1989, McCarthy, Lewis, et al. 1995). Even though a definitive diagnosis of ligamentous anomalies is rarely described, AA instability with a normal radiographic appearance of the dens suggestive of ligamentous dysfunction is not infrequent (McCarthy, Lewis, et al.
Definitive diagnosis of such anomalies often requires post-mortem examination and therefore is not attainable in most cases. The origin of ligamentous dysfunction also remains uncertain. A predisposition to traumatic rupture is suspected to be the most common cause of ligamentous instability, although this hypothesis has yet to be proven.

A recent anatomical study identified fibrocartilage at the insertion points of the ligaments supporting the AA joint (enthesis fibrocartilage) (Kupczynska, Wieladek, et al. 2012). Large chondrocytes were also identified physiologically in the central region of the transverse ligament (sesamoid fibrocartilage) and dorsal AA ligament in large breed dogs. The authors of that paper hypothesized that a pathological process affecting the cartilaginous components of the ligament could theoretically predispose dogs to ligamentous rupture. This etiology is commonly recognized in humans suffering from rheumatoid arthritis, resulting in an immune-mediated impairment of the enthesis cartilage and commonly resulting in craniocervical junction instability.

There is increasing evidence that genetic predisposition to AA instability exists in dogs. The susceptibility of toy breed dogs has been known for decades and suggests a genetic etiology (McCarthy, Lewis, et al. 1995, Plessas & Volk 2014). Recently, 4 large breed dogs reported with pituitary dwarfism due to LHX3 mutation also had evidence of incomplete ossification of the atlas and AA instability (Voorbij, Meij, et al. 2015). Another recent study identified familial anomalies of the odontoid process in 7 of 19 related Standard poodles assessed radiographically (Stigen, Aleksandersen, et al. 2013). Based on pedigree analysis, an autosomal transmission was considered likely involved in Standard poodles suffering from dens congenital malformations.

Other etiologies infrequently associated with AA instability include incomplete ossification of the atlas and cervical block vertebrae causing a fulcrum effect on the craniocervical junction (Lin & Coolman 2009, Warren-Smith, Kneissl, et al. 2009, Parry,
Pure traumatic injuries of the AA joint can also be encountered. Cervical fracture or luxation is observed in dogs after high energy trauma such as collisions (vehicles or stationary objects), high velocity projectile (gunshot) or bite wounds. The craniocervical junction is the most common site of traumatic injury which is thought to be due to a fulcrum effect generated on C2 by the weight of the head cranially and musculotendinous attachments caudally (Fig I.3) (Dewey 2013). An extensive review of the pathogenesis of cervical traumatic injuries is beyond the scope of this manuscript. However, it should be considered that when C2 fracture occurs at the level of the dens, it results in AA instability with similar biomechanical characteristics as observed with congenital malformation of the dens or its ligaments.

**Epidemiology**

AA instability is typically reported in young adults, typically less than 2 years old, actually 52-70% of cases younger than 1 year old, with a mean age of 21.3 months and no sex predilection identified (Cerda-Gonzalez & Dewey 2010, Plessas & Volk 2014).

The most common types of dog affected by AA instability are, by far, the toy breed dogs. A recent review reported that Yorkshire terriers, Toy poodles and Chihuahuas represented 28%, 17% and 15% of cases respectively and had an overall mean body weight of 3.6kg (Plessas & Volk 2014). Other types of dogs, mostly small breeds, can be also affected, with 38 different breeds identified in that same report. Larger dogs have uncommonly be reported, including Doberman pinschers, Basset hounds, Weimaraners, Saint-Bernards, Rottweilers, Standard poodle and German shepherd (Hurov 1979, Read, Brett, et al. 1987, Watson, de Lahunta, et al. 1988, Huibregtse, Smith, et al. 1992, Wheeler 1992, Rochat & Shores 1999, Patton, Almes, et al. 2010, Stigen,
Aleksandersen, et al. (2013). The overrepresentation of miniature breeds is attributed to their suspected predisposition to AA congenital malformations. However, it could be argued that their small size predisposes them to more severe injuries as human companion animals (collisions, fall from furniture, altercations with larger dogs…).

**Acute and chronic spinal cord and brainstem injury**

Acute and chronic dorsal displacement of the axis results in various degrees of traumatic spinal cord injury (SCI) and caudal brainstem injury. These injuries are typically subdivided into primary and secondary injuries.

Primary injuries represent direct parenchymal damage caused by concussion, compression, shear, laceration, distraction or contusion. These causes result in intraparenchymal and extraparenchymal lesions, as hemorrhage, neuronal cell body and axonal injury, and dural tear (DiFazio & Fletcher, 2013). Slow compressive injuries present clinically in a very different way than acute high energy traumatic injuries. Typically, chronic compressive lesions are better tolerated in dogs and primarily result in demyelination, which can be potentially reversible (Fingeroth, Forterre, et al., 2015). This type of injury is observed after chronic subluxation of C2 causing constant compressive forces on the spinal cord. However, this type of lesion is probably not the most common cause of clinical presentation. Given the high degree of motion and stress occurring at the craniocervical junction, repetitive concussive and contusive injuries are likely to occur. The velocity at which such injuries occur will depend on the degree of instability and the velocity of the head and neck motions. Therefore, the severity of SCI can be highly variable and unpredictable. This correlates with clinical observations that severe SCI can occur within seconds but that less severe presentation can be observed for months to years without aggravation.
Secondary injuries are the result of a cascade of vascular and biochemical events that aggravates the primary injury and locally extend the damage within the central nervous system. A complete description of these mechanisms is beyond the scope of this review. Briefly, it involves accumulation of glutamate, intracellular influx of sodium, cytotoxic edema, intracellular influx of calcium and free radical production which exacerbates the injuries and release of inflammatory mediators (DiFazio & Fletcher 2013). The secondary injuries are important in the pathogenesis of caudal brainstem injury observed in AA instability and likely occur via local extension of the primary SCI. Brainstem damage can also be observed secondary to iatrogenic SCI during surgical correction of AA instability. The proximity of the surgical site with the brainstem (containing the cardiorespiratory centers) likely plays a significant part in the high intraoperative and immediate postoperative mortality associated with stabilization (Plessas & Volk 2014).

Clinical presentation and neuroanatomical localization

Clinical signs are, in most cases, consistent with a lesion localized to the cranial cervical spinal cord segments. The first 3 cervical spinal cord segments are located within the craniocervical junction and contain descending upper motor neurons for the entire body (from neck to tail) as well as ascending sensory pathways. Therefore, various degrees of weakness (tetraparesis/plegia, respiratory compromise, inability to lift the head) and ataxia (most commonly proprioceptive) can be observed. Neck pain is also commonly identified (53-77% of cases), although neck mobilization is often not performed in those suspected cases due to risk of aggravating the AA subluxation and causing sudden death (Cerda-Gonzalez & Dewey 2010, Parent 2010). Typically, the presence of proprioceptive ataxia and tetraparesis with appropriate mentation, cranial

As stated previously, the progression of the symptoms can vary from hyperacute (including sudden death from respiratory arrest) to chronic (slow to non-progressive) depending on the severity of the instability. Episodic pain and/or weakness with normal neurological examination between episodes has been also reported, with owners easily confusing such episodes with epileptic seizure activity (Stigen, Aleksandersen, et al. 2013). Cerebellovestibular clinical signs including hypermetria, imbalanced gait (vestibular ataxia), head tilt, absent menace response, positional strabismus and nystagmus can occasionally be observed (Parent 2010, Dewey, Marino, et al. 2013, Dewey 2013). Most of these signs will be observed when a concomitant caudal occipital malformation or atlanto-occipital overlapping is present. However, hypermetric gait and vestibular ataxia can be present solely with AA instability, presumably due to impairment of the vestibulospinal and spinocerebellar tracts (Dewey 2013). The presence of concomitant syringomyelia can also confuse the localization of the lesion by affecting multiple regions of the spinal cord including lower cervical and thoracolumbar (Dewey 2013).
IV. Diagnosis of atlantoaxial instability

Differential diagnoses and diagnostic strategy

In most cases, the signalment and neurolocalization provides strong clinical indication that a craniocervical junction anomaly is responsible for the clinical signs. Such anomalies include caudal occipital malformations (Chiari-like malformation), atlanto-occipital overlapping, AA instability, AA dorsal fibrous band, and/or occipitoatlantoaxial malformations (Cerca-Gonzalez & Dewey 2010). These conditions can also predispose dogs to syringomyelia, thought to be due to chronic caudal fossa overcrowding and disruption of the normal cerebrospinal fluid flow (Cerca-Gonzalez, Olby, et al. 2009). Other common disorders of young toy breed dogs that should be considered include meningoencephalomyelitides of unknown origin (immune-mediated), intervertebral disc herniations, other cervical congenital anomalies (such as arachnoid diverticulum, vertebral malformations) and exogenous traumatic spinal cord injuries (Sharp & Wheeler 2005, Cerdà-Gonzalez & Dewey 2010). In more atypical clinical presentations (cerebellovestibular signs or episodic collapse), cerebrovascular accidents, metabolic disorders (such as portosystemic shunt or hypoglycemia) and systemic intoxication should also be considered (Dewey 2008). Even though uncommon in younger dogs, spinal or intracranial neoplasia can cause similar clinical signs if affecting the craniocervical junction (Westworth & Sturges 2010). Differential diagnoses considered will vary upon the exact signalment, presentation and the extent of neurological deficits observed.

In most cases, AA instability is diagnosed via imaging of the craniocervical region, either by demonstrating subluxation or an abnormal odontoid process (Cerca-Gonzalez & Dewey 2010, Dewey, Marino, et al. 2013, Stalin, Gutierrez-Quintana, et al. 2015). One
of the limitations to that diagnostic approach is that the degree of instability
demonstrated via imaging does not always correlate with the severity of clinical signs.
Recently, in a prospective study, several Standard poodles showed radiographic
evidence of abnormal odontoid processes while most of them were either asymptomatic
or had an history of “seizure-like” episodes but no neurological deficits upon examination
(Stigen, Aleksandersen, et al. 2013). The prevalence of asymptomatic anomalies in toy
breed dogs is unknown, but clinicians should keep in mind the possibility of identifying
subclinical anomalies in a dog suffering from another unrelated condition. This implies
that other diseases listed in the differential diagnoses should be ruled out if possible
before diagnosing AA instability. On the other hand, AA instability may be difficult to
identify with standard imaging techniques. Some cases may have normal vertebral
anatomy and the evidence of instability may not be visible when using standard
positioning.

To increase diagnostic sensitivity, some authors have recommended obtaining
slightly flexed views to further demonstrate instability (Westworth & Sturges 2010). This
dynamic assessment is controversial as it can result in acute worsening of spinal cord
injury and even sudden death. Fluoroscopy has also been reported to control more
precisely the degree of flexion applied through continuous visualization of the vertebrae
human medicine for the diagnosis of AA instability associated with Down syndrome.
Historically, dynamic studies have been used to objectively determine the level of
instability, however, a recent publication raises concerns about this practice and
suggests an alternative method of measurement using radiographs obtained in neutral
Taking those limitations into consideration, most of the recent reviews on AA instability recommended performing advanced imaging, such as computed tomography and/or magnetic resonance imaging, in order to safely document any evidence of instability and to investigate the possibility of concurrent diseases primarily causing or exacerbating the clinical signs (Cerda-Gonzalez & Dewey 2010, Dewey, Marino, et al. 2013, Stalin, Gutierrez-Quintana, et al. 2015). However, to the author’s knowledge there are currently no studies comparing the sensitivity and specificity of the different imaging modalities.

**Radiography of the atlantoaxial joint**

Radiography is usually the first line of imaging modality used to diagnose AA instability. Orthogonal views of the craniocervical region in neutral position are diagnostic in many cases (Westworth & Sturges 2010). Diagnosis can generally be reached either by demonstrating the subluxation (dorsal displacement of C2), or by identifying the aplasia, hypoplasia or fracture of the odontoid process. Radiographic evidence of dens dysplasia has been reported in 56% of cases (Plessas & Volk 2014).

Abnormal displacement of C2 is best appreciated by measuring the distance between C1 dorsal arch and the cranial border of C2 spinous process (Fig I.4). A radiographic study reported that normal small breed dogs (<15kg) demonstrated an overlap of 0.56 ± 0.35cm and as low as 0.21 ± 0.16cm in Cavaliers King-Charles Spaniels (Stalin, Rusbridge, et al. 2008). Based on these measurements significant separation between the C1 dorsal arch and C2 spinous process would strongly suggest AA instability. Anatomically, this can be explained by a rupture of the dorsal AA ligament, that is normally very short. That study did not establish a cutoff value for what would be considered abnormal overlapping. However, a decreased overlap of more than 3mm
from neutral to flexed position was considered abnormal in that study (Stalin, Rusbridge, et al. 2008). Based on these results, measuring a distance larger than 3mm between the C1 dorsal arch and C2 spinous process would be suggestive of AA instability (both in neutral or flexed position). This cutoff value is extrapolated from data obtained in normal dogs and further investigation is necessary to determine its diagnostic value (sensitivity and specificity), but, in the meantime, could be used as a general guideline. Currently that distance remains subjectively interpreted as increased or within normal range (Stalin, Gutierrez-Quintana, et al. 2015).

**Fig I.4** Radiographic interpretation of the distance (d) between C1 dorsal arch and C2 spinous process measured on lateral radiographs.

The dorsal border of the vertebral foramen of C2 is used for reference (red line). The first perpendicular line is placed at the level of the caudal border of C1 dorsal arch (blue line), the second perpendicular line is placed at the level of the cranial border of the C2 spinous process (green line). The distance is measured along the reference axis (yellow line). (A) Radiograph demonstrating the typical increased distance observed with atlantoaxial instability (d=5mm). (B) Radiograph representing an absence of overlap (d=1.5mm) which may be positional rather than pathological.
Computed tomography of the atlantoaxial joint

Computed tomography (CT) provides a detailed, 3 dimensional depiction of the position and conformation of the vertebrae (Sorjonen & Shires 1981). CT can be combined with myelography to assess spinal cord compression secondary to vertebral subluxation. However, myelography carries risks of triggering seizure activity which are typically transient and well tolerated in normal dogs but could have disastrous consequences in dogs suffering from AA instability (Kishimoto, Yamada, et al. 2004). The major advantage of CT versus other imaging modalities is that it allows for an accurate description of spatial relationships and therefore can be used to determine ideal placement of surgical implants preoperatively (Vizcaino Reves, Stahl, et al. 2013, Stalin, Gutierrez-Quintana, et al. 2015). It is also very sensitive to detect abnormal osseous structures such as an abnormal odontoid process, occipitoatlantoaxial dysplasia, caudal occipital malformations or incomplete ossification of the atlas (Rylander & Robles 2007, Cerda-Gonzalez & Dewey 2010, Parry, Upjohn, et al. 2010). CT can also be used to determine implant position postoperatively to assess the adequacy of the surgical stabilization, as it has much greater sensitivity than conventional radiographs to detect vertebral canal violation (93.4% vs. 50.7%) (Hettlich, Fosgate, et al. 2010). The major limitation of this imaging modality is that it provides minimal contrast within soft tissues and therefore does not allow visualization of many intraparenchymal lesions, such as syringomyelia (early stages) and inflammatory diseases. For that reason magnetic resonance imaging is usually preferred for diagnostic purposes due to its superior ability to detect other concomitant diseases (Cerda-Gonzalez & Dewey 2010, Dewey, Marino, et al. 2013).
Magnetic resonance imaging (MRI) allows precise visualization of the degree of spinal cord compression due to good soft tissue contrast. Even though spatial resolution is reduced compared to CT, AA subluxation and odontoid process pathologies are easily identified on MRI (Middleton, Hillmann, et al. 2012). A recent study reported the use of MRI to assess the integrity of ligamentous structures supporting the AA joint in dogs (Middleton, Hillmann, et al. 2012). That study described the normal appearance of the AA ligaments as being of low intensity on all pulse sequences (iso- or hypointense to muscle tissue). The longitudinally directed ligaments, apical and dorsal AA, were better visualized in the sagittal plane while the transverse ligament was better visualized in the transverse plane. The alar ligaments were more difficult to visualize and required a reconstructed plane at 20° from dorsal using thin slices (0.6mm) to be visualized and were identified in 9/10 cases. This report also included the MRI evaluation of 3 dogs suffering from AA instability. Anomalies identified on MRI in those dogs included thickened alar ligaments, elongated and irregular apical ligament and absent transverse ligament (Middleton, Hillmann, et al. 2012). Further investigation is necessary to determine the diagnostic value of ligamentous assessment in dogs. Nevertheless, MRI is currently the main imaging modality available for the evaluation of ligamentous integrity and may be worthwhile to perform when gathering evidence of instability using neutral positioning. In human medicine, assessment of ligamentous structures is routinely performed using high-resolution magnetic resonance imaging and the identification of ligament rupture is often considered in therapeutic decision making of craniocervical instabilities (Krakenes & Kaale 2006, Menezes & Traynelis 2008).

In veterinary medicine, MRI is considered the gold standard to assess intramedullary lesions in vivo and more specifically to identify acute spinal cord injury. Intramedullary
edema and gliosis can be observed secondary to acute and chronic spinal cord injury. These lesions are depicted on MRI as an intramedullary hyperintensity on T2 weighted images (Westworth & Sturges 2010, Middleton, Hillmann, et al. 2012). The extent of intramedullary hyperintensity has been correlated with prognosis for recovery in intervertebral disc herniation but further investigation is necessary to determine if this result can be applied to other causes of spinal cord injury (Ito, Matsunaga, et al. 2005). MRI is very sensitive to detect hemorrhagic and fluid-filled lesions, whether they are intramedullary, intradural-extramedullary or extradural (Kent, Eagleson, et al. 2010, Forterre, Vizcaíno Reves, et al. 2012b). Syringomyelia is also mainly investigated through use of MRI, and overall MRI is usually considered the gold standard for investigation of most craniocervical junction anomalies in dogs (Cerda-Gonzalez & Dewey 2010, Driver, De Risio, et al. 2012, Dewey, Marino, et al. 2013, Ives, Doyle, et al. 2015).

**Other imaging modalities of the atlantoaxial joint**

Ultrasound technique has been described for the visualization of the craniocervical region of dogs and cats. Current use of this modality have included assessment of the caudal fossa and cerebellar herniation associated with caudal occipital malformations, assessment of elevated intracranial pressure via Doppler pressure measurement of the basilar artery and ultrasound assisted cerebrospinal fluid collection from the cisterna magnum (Schmidt, Wigger, et al. 2008, Duque, Dominguez-Roldan, et al. 2011, Etienne, Audigie, et al. 2015). Similar techniques could be adapted to investigate the AA interrelationships and in particular the presence and positioning of the odontoid process within the vertebral canal. In human medicine, ultrasound is routinely used in prenatal and neonatal detection of spinal congenital malformations (Ladino Torres & DiPietro
The spinal canal anatomy is best visualized early in the infantile development due to incomplete ossification of the vertebrae. A similar approach could be applied for screening purposes in toy breed dogs, however further investigation is necessary to determine whether the degree of ossification at birth prevents ultrasonic assessment.

As stated previously, myelography and fluoroscopy have been reported anecdotally to demonstrate spinal cord compression and dynamic instability (Cerda-Gonzalez & Dewey 2010). Those techniques both potentially carry risks of aggravation of an already existing spinal cord injury (either via uncontrolled convulsions or via overzealous flexion of the AA joint). Such risks are difficult to justify given the availability of safer methods of investigations.
V. Medical management of atlantoaxial Instability

Indications for medical management

There seems to be some general consensus that medical management should be reserved for cases presenting with only mild AA instability causing minimal to no neurological deficits and considered unlikely to cause acute spinal cord injury. However, current diagnostic tools and assessment methods do not provide accurate information in order to follow that guideline. Even if evidence of instability can be demonstrated, there is no established grading system correlating imaging findings with severity of neurological status or predicting the probability of future deterioration or relapse. Similar limitations exist in human medicine where the prevalence of asymptomatic instability can be high with certain pathologies, such as Down syndrome or os odontoideum. Even though some debate exists, the most common approach in human medicine is to reserve conservative management for cases without neurological deficits (Klimo, Kan, et al. 2008, Menezes 2008b). Other factors, such as type and level of physical activity, are also considered in the treatment selection in humans, but overall good outcome can be obtained with conservative management in asymptomatic cases (Klimo, Kan, et al. 2008, Menezes 2008b, Arvin, Fournier-Gosselin, et al. 2010, El-Khoury, Mourao, et al. 2014).

If a similar approach was followed in veterinary medicine, most dogs presented for AA instability would be good candidates for surgical stabilization, as they rarely are asymptomatic. There are, however, many reasons why a more conservative approach is often elected. An obvious reality in veterinary medicine is that financial constraint is a major reason for pet’s owners to elect conservative therapy instead of surgery. Probably the most relevant justification is that the surgical techniques have been associated with significant intraoperative and immediate postoperative mortality rates (5-8%) (Plessas &
Volk 2014). Mortality is attributed to iatrogenic acute spinal cord injury that can occur with manipulation of the AA joint in the perioperative and intraoperative period or with inappropriate placement of the implants used for stabilization. Other limitations of surgical techniques, such as poorly ossified vertebrae in very young dogs or the limited experience of the surgeon, are also taken in consideration and may justify a conservative approach. Limitations and success rates of surgical stabilization are further discussed later. Overall, clinicians have to consider that the success rate of surgery may be similar or even inferior to medical management in selected patients given that some dogs seem to remain asymptomatic (Patton, Almes, et al. 2010, Middleton, Hillmann, et al. 2012, Stigen, Aleksandersen, et al. 2013).

There is very limited available data on medical management of AA instability in dogs, likely because the disease has been considered a surgical condition since very early descriptions (Geary, Oliver, et al. 1967). Most of the current literature has been focused on the description of various surgical methods in an attempt to improve outcome. There is only 1 case series published describing long-term follow up of 16 medically managed dogs affected by AA instability (Havig, Cornell, et al. 2005). This study concluded that the best candidates for medical management were dogs presenting for a single acute episode of less than 1 month duration. Interestingly, the severity of neurological deficits was not associated with success rate.

**Conservative management principles and recommendations**

Modern theories on stabilization systems of the spine include 3 major physiological components (Panjabi 1992a). Firstly, the passive musculoskeletal subsystem including all of the passive supporting anatomical structures of the spine (vertebrae, ligaments, intervertebral discs and synovial joints) as well as epaxial muscles in their passive state.
Secondly, the active musculoskeletal subsystem containing epaxial muscles and tendons whose biomechanical properties change over time depending on the degree of muscle contraction. And, finally, the neural and feedback subsystem (or control system) involving proprioceptive receptors and central nervous system integration centers that controls and regulates the active subsystem. Even though most biomechanical research is focused on the passive subsystem, the other 2 systems cannot be ignored as they both play a major role in maintaining spinal stability in vivo. The importance of these systems could partially explain why dogs and humans with radiologic evidence of instability are able to compensate and may remain asymptomatic their whole life. Actually, in human medicine, increased muscle tone is thought to improve spinal stability and is the basis for muscle support therapy for chronic instabilities such as lower back pain syndrome (Panjabi 2003).

The conservative management of AA instability aims at restoring the compensatory mechanisms of the spine to maintain a functional degree of stability. Following an acute event (sudden movement or trauma) the level of spinal instability can increase beyond the compensatory capacity of the spine. The main objective of conservative management is to provide external support of the head and neck for a period of time estimated sufficient to allow the soft tissue injuries (such as ligament / articular capsule stretch or tendon / muscle injury) to heal and for the development or restoration of the compensatory mechanisms. There is actually little evidence demonstrating that immobilization of the neck successfully reduces strain on the AA joint or that it eventually achieves an improved level of stability. However, it is considered common knowledge that reducing the range of motion in any joint will allow healing of partially damaged soft tissue and formation of fibrous scar tissue to stabilize the joint.

Many different methods of external support of the cervical spine have been used for
AA instability or vertebral fracture/luxation. Various materials have been used, such as a cervical splint (cardboard, plastic, metallic, plaster), while some clinicians prefer to use soft padded bandages without splint material (Schulz, Waldron, et al. 1997, Hawthorne, Blevins, et al. 1999, Havig, Cornell, et al. 2005, Sharp & Wheeler 2005). The type of bandage used is clinician dependent as no study is available to compare their effectiveness. However, in order to support the AA joint effectively, the bandage should offer at least some support of the head given that the head’s weight is considered a major source of strain on the AA joint (Reber, Burki, et al. 2013). A bandage that would only limit cervical mobility without supporting the head could theoretically increase the fulcrum effect exerted on the AA joint. Therefore, splints should extend from the rostral region of the mandible to the sternum ventrally and from the caudal border of the orbit to the thoracic region dorsally. In one retrospective study on non-surgical management of AA instability, splints were maintained for 4-15 weeks (mean and median of 8.5 weeks) (Havig, Cornell, et al. 2005).

Strict exercise restriction, avoiding any activity involving strain on the head, is also emphasized with progressive return to normal activity level after splint removal. Pain management is also prescribed as required by the patient. This would commonly include opioids, gabapentin, pregabalin and non-steroidal anti-inflammatories. The use of corticosteroids is also commonly reported to treat hypothetical inflammatory components of the spinal cord injury. Other medications used in medical management of AA instability have included treatments for central nervous system vasogenic edema such as mannitol; muscle relaxants such as methocarbamol, diazepam or acepromazine; and gastrointestinal prokinetics such as cimetidine (Havig, Cornell, et al. 2005). The reported use of muscle relaxants is empirical and aimed at relieving pain from muscle spasms; however clinicians should consider the possibility that increased muscle tone may provide compensatory stability, which raises the question whether they should be
prescribed for AA instability.

Success rates and limitations

As stated previously, very limited data is available assessing the success rate of medical management in dogs. A large proportion of medically managed cases in the literature are described as individual case reports which constitute inappropriate data to assess outcome (Downey 1967, Gilmore 1984, Hawthorne, Cornell, et al. 1998, Lin & Coolman 2009). The best level of evidence is provided by a retrospective analysis of 16 cases with long term follow-up (12 months) (Havig, Cornell, et al. 2005). The authors reported an overall success rate of 62.5% (10/16), with only 37.5% (6/16) of dogs being ambulatory prior to therapy. Dogs that did not respond to therapy relapsed within the 12 month period and either died or were euthanized, resulting in a mortality rate of 37.5% (6/16).

In general, the most significant prognostic factor in canine spinal cord injury is the presence or absence of nociception. However, this factor is of little clinical value in the cervical region as loss of nociception would imply respiratory arrest and death. To date, the main positive predictive factor that has been identified for medical management of AA instability was a short duration of clinical signs (<30 days) (Havig, Cornell, et al. 2005).

The most common complication associated with conservative management is relapse or clinical deterioration due to inappropriate stabilization (Havig, Cornell, et al. 2005). Other reported complications are mostly related to the cervical splints or bandages and may include respiratory difficulties (excessively tight bracing), difficulty swallowing and aspiration pneumonia (overextended neck), cutaneous and ear canal complications (dermatitis, otitis, abrasions, decubital ulcers), corneal ulcers (direct
contact with bandage) and general discomfort (poorly tolerated bandage) (Schulz, Waldron, et al. 1997, Havig, Cornell, et al. 2005, Sharp & Wheeler 2005). In one study, complications were reported in 44% of cases (7/16) (Havig, Cornell, et al. 2005).
VI. Surgical stabilization of the atlantoaxial joint

Indications for surgical stabilization

There is currently insufficient data both in veterinary and human medicine to formulate objective decisional criteria for AA stabilization. The major indication for surgical spinal stabilization is the establishment of sufficient evidence to confirm that a non-functional instability is present (i.e. beyond spinal compensatory mechanisms). This implies the demonstration of evidence of AA instability on imaging as well as providing evidence that the instability is causing clinical signs.

In human medicine, the presence of neurological deficits is generally necessary to justify surgical therapy (Menezes 2008b). However, some authors have argued that radiological evidence of instability is the minimum criterion (Klimo, Kan, et al. 2008). For instance, in the condition named os odontoideum (separated dens), the presence of an abnormal odontoid process can be considered enough evidence to support stabilization. It should be emphasized that this statement was strongly justified by the high surgical success rate and almost a 100% fusion rate with the current surgical techniques. In that regard, the success rate of surgical AA stabilization is significantly different in canine patients and therefore a more conservative approach may be indicated. A surgical success rate is not yet fully established in dogs, although a general trend towards improvement is seen in the literature (Table I.1). Nevertheless, it is generally accepted that many dogs referred for AA instability are surgical candidates given that they often present with significant neurological deficits. This is reflected by the fact that in the past 50 years, most of the literature on the subject has been focused on developing and improving techniques for AA stabilization (LeCouteur, McKeown, et al. 1980, Sorjonen & Shires 1981, Kishigami 1984, Schulz, Waldron, et al. 1997). A fairly conservative
approach would be to consider surgical stabilization in cases where instability is demonstrated and having presented clinical signs for more than a month (Havig, Cornell, et al. 2005).

**General considerations on cervical spine stabilization**

The objective of spinal stabilization is to restore the normal function of the spine, which is to provide flexible support of the body without significantly impairing the vital structures traveling through the vertebral canal and intervertebral foramen. From a biomechanical perspective, this equates to bringing the pathologically increased neutral zone of a functional spinal unit within the pain free zone (Panjabi 1992b). Theoretically, this can be achieved through dynamic systems such as joint and intervertebral disc replacements, which are a major focus of current spinal biomechanics research (Zang, Liu, et al. 2015). However, in current practice, stabilization is mostly achieved through rigid fixation of the affected motion unit to obtain bony fusion (arthrodesis or fracture healing). Because bony fusion is the main goal, instrumentation constructs with rigid biomechanical characteristics (high stiffness, minimal range of motion) are usually preferred. One significant limitation of this method is that the resultant reduction in local spinal flexibility of that vertebral motion unit may generate fulcrum effects, causing deterioration of adjacent motion units.

Because of the very irregular and non-linear structure of the spine, contouring is often necessary to use bone plates and locking plate constructs. This is a major limitation for compression plates, because good contact between bone surface and the plate is necessary to reach rigid stabilization. The locking systems are less affected by this limitation as contact with bone surface is not absolutely required (Barnhart, Rides, et al. 2013). However, the spinal anatomy provides limited numbers of bone corridors for screw insertion and therefore contouring is still necessary in many cases. In that regard, cemented constructs are less technically demanding since the cement can be molded around the screws or pins and adapted to most implant placement configurations. Biomechanical studies comparing different cemented constructs have suggested they can achieve a similar level of rigidity, however direct comparison with plate fixation is lacking (Garcia, Milthorpe, et al. 1994, Hicks, Pitts, et al. 2009, Agnello, Kapatkin, et al. 2010, Hettlich, Allen, et al. 2013).

misplacement can still be infrequently identified on postoperative CT (4.4-7%), although only a small portion of those are considered clinically significant (0.25-0.34%) (Elliott, Tanweer, et al. 2012).

In canine patients, cervical spine implant placement is known to be challenging, particularly when using a ventral approach. A recent cadaveric study using previously reported landmarks and angles of insertions for C4 and C5 vertebrae obtained high rates of vertebral canal violation (up to 100% when using bicortical pedicular pins) (Hettlich, Allen, et al. 2013). Similar results had previously been reported with the placement of C4 and C5 pedicular implants, violating the vertebral canal in 62.5% of cases, in contrast with screws positioned within the transverse process, causing no violation (Hicks, Pitts, et al. 2009). The use of monocortical screws placed in the vertebral body is an alternative and has led to a decrease in the rate of vertebral canal violation in a cadaveric study (9.7%) (Hettlich, Allen, et al. 2013). However, the concern remains, using this method, that inadvertent bicortical drilling can result in dramatic spinal cord injury as the drill is oriented toward the spinal cord.

The rate of vertebral canal violation occurring in clinical cases of cervical stabilization is unknown in dogs and is likely underestimated by clinicians given the low sensitivity of postoperative radiographs compared to CT (Hettlich, Fosgate, et al. 2010). Compared to human medicine, the methods used for surgical planning and to ensure adequate placement of cervical implants are very limited in canine patients. There is some evidence suggesting that use of intraoperative fluoroscopy may significantly improve implant placement accuracy, however its application can be difficult in the cervical region due to very oblique positioning of the implants and close proximity to the vertebral canal (Wheeler, Cross, et al. 2002).

The available anatomical studies describing optimal safe implantation corridors in
dogs are based on CT images, although similar studies are lacking for most implantation corridors of the AA joint. Values of transverse projected angles measured from the ventrodorsal axis for pedicular implants have been reported from C2 to C7 and ranged from 30° to 60° depending on the vertebra (Watine, Cabassu, et al. 2006, Corlazzoli 2008). These studies have suggested that attempting to place cervical pedicular implants without causing some degree of vertebral artery injury is probably unrealistic, as safe implantation corridors are extremely narrow. Another consideration is that significant individual variation of projected angle values (range width of 10-15°) exists in dogs. This likely precludes the use of reported mean values to target narrow implantation corridors and therefore individual preoperative planning should be performed whenever possible. Even if more precise optimal safe implantation corridors can be defined, accurate implant placement is difficult and new methods of implant placement are necessary to achieve the level of accuracy required for ventral cervical spine stabilization in dogs.

**Surgical approaches and reduction methods of the atlantoaxial joint**

The surgical approach to the AA region is generally selected based on surgeon’s preferences and elected method of stabilization. Two types of approaches are used respectively for ventral and dorsal stabilization techniques. The dorsal approach has been historically preferred likely due to a lower risk of damaging important vital structures located ventrally (trachea, esophagus, carotid sheaths…) (Geary, Oliver, et al. 1967, Chambers, Betts, et al. 1977, LeCouteur, McKeown, et al. 1980, Jeffery 1996). However, a significant advantage of the ventral approaches is that it provides exposure to the broad vertebral structures (C1 lateral masses, C2 vertebral body) offering greater bone purchase for the implants (Sorjonen & Shires 1981, Schulz, Waldron, et al. 1997).
Ventral approaches also provide access to the AA synovial joint, allowing the surgeon to perform standard arthrodesis techniques (burring of articular cartilage and/or bone grafting) which may improve subsequent fusion rates (Festugatto, Mazzanti, et al. 2013).

The AA dorsal approach is performed through the dorsal raphe median allowing exposure of the C1 dorsal arch and C2 spinous process (Funkquist 1962, Piermattei & Johnson 2004). For most dorsal AA stabilization techniques, exposing the vertebral canal is required. This is achieved through incision of the interarcuate ligament and dorsal atlantooccipital membrane to allow positioning of stabilizing material under the dorsal arch of C1. It is noteworthy that such implants are always positioned within the subarachnoid space because the dura mater is tightly attached to the C1 dorsal arch, and therefore cerebrospinal fluid leakage is expected.

Traditionally, the ventral approach of the AA joint has been performed through midline dissection (Sorjonen & Shires 1981, Piermattei & Johnson 2004). Blunt dissection of the connective tissue allows lateral retraction of the larynx, trachea, esophagus and carotid sheaths to access the hypaxial muscles. However, the right sternothyroideus muscle has to be incised using this approach. An adaptation of this traditional approach was described using a paramedian dissection between the sternocephalicus and sternothyroideus muscles (Shores & Tepper 2007). Using this method both carotid sheaths are retracted to the left side with the other vital structures.

Using a dorsal approach, the spinous process can easily be used to hold C2 and reduce the subluxation while positioning the stabilizing implants. In contrast, when a ventral approach is used, the vertebral body of C2 has a tendency to move craniodorsally (away from the surgeon) which renders reduction and implant positioning challenging. Several reduction methods have been reported in the literature to overcome this problem. Direct methods of reduction include the use of articular distractors,
orthopedic reduction forceps, or placing monocortical bone screws to apply caudoventral traction using suture material (Platt, Chambers, et al. 2004, Sharp & Wheeler 2005, Dewey 2013). An indirect method was also described using a Gelpi retractor placed between the base of the occipital bone (intercondyloid incisure) and the C2-3 intervertebral disc (fenestrated beforehand) to apply craniocaudal distraction (Forterre, Vizcaino Reves, et al. 2012a).

**Surgical stabilization techniques of the atlantoaxial joint**

In the past 50 years, many AA stabilization techniques have been described and new modifications continue to be developed regularly. Geary et al. described the very first attempt at surgical stabilization of the AA joint consisting of placing a metallic wire between the C2 spinous process and the wings of C1 (Geary, Oliver, et al. 1967). This rapidly proved to be ineffective and the technique was modified by placing the wire under the dorsal arch of C1 instead (Geary, Oliver, et al. 1967). From 1967 to 1981, the dorsal suture technique was standard of care even though the exact method of wire fixation could vary as well as the type of suture material involved (metallic vs. non-metallic) (Geary, Oliver, et al. 1967, Gage & Smallwood 1970, Chambers, Betts, et al. 1977, Lakatos, Suter, et al. 1981). In 1980, Lecouteur et al. described 4 cases stabilized through a dorsal ligamentoplasty consisting of looping each half of the nuchal ligament under the dorsal arch of C1 and then secured against the spinous process of C2 (LeCouteur, McKeown, et al. 1980). This method was not subsequently repeated and dorsal suture techniques became less frequently reported in the following years (Denny, Gibbs, et al. 1988, Thomas, Sorjonen, et al. 1991, Beaver, Ellison, et al. 2000).

In 1979, a ventral approach to the AA joint had only been performed to stabilize a fracture of the axis using bone plates or Steinman pins with polymethylmethacrylate
(PMMA) cement (Rouse 1979). In 1981, Sorjonen and Shires described the first ventral technique for stabilization of congenital AA subluxation using 2 transarticular pins. This publication provided a detailed technical description of the AA transarticular arthrodesis which is still considered a reference to date (Sorjonen & Shires 1981). In 1988, Denny et al. modified this technique using the same implant positioning but used lag screws instead of pins (Denny, Gibbs, et al. 1988). From 1988 to 2000, more than 70 cases were reported using variations of the transarticular fixation techniques in multiple case reports and case series (Denny, Gibbs, et al. 1988, Johnson & Hulse 1989, Thomas, Sorjonen, et al. 1991, Wheeler 1992, Rochat & Shores 1999, Beaver, Ellison, et al. 2000). The lag screw transarticular technique remains one of the most commonly used AA stabilization technique to this day, even though case series have been less frequently reported in the past decade (Shores & Tepper 2007, Jeserevics, Srenk, et al. 2008).

In 1997, Schulz et al. described the first ventral multi-implant technique with PMMA cement embedding (Schulz, Waldron, et al. 1997). They combined 6 pin positions including 2 transarticular pins, 2 pins located in the lateral masses of the atlas and 2 pins in the caudal vertebral body of the axis, all held together with PMMA. Since then, numerous variations of this technique have been described. In 2002, Knipe et al. described a 4 screw technique with PMMA (2 in atlas/ 2 in axis) (Knipe, Sturges, et al. 2002). In 2004, Platt et al. described a technique using 3 screws in the atlas, 2 screws in the axis combined with transarticular pins and PMMA (Platt, Chambers, et al. 2004). In 2004, Sanders et al. proposed a modified 6 screw technique using cerclage wire and Steinman pins in addition to the PMMA cement to increase the rigidity of the montage (Sanders, Bagley, et al. 2004). In 2013, Aikawa et al. presented the results of a large case series using an elaborate adaptable strategy using 9 possible implantation sites. Exact implant positioning was elected intraoperatively based on the quality of bone
purchase obtained with 6 standard implant sites (2 transarticular, 2 in C1 lateral masses, 2 in C2 caudal vertebral body). Most patients were stabilized using 6 or 7 threaded pins embedded in PMMA (Aikawa, Shibata, et al. 2013). Over the past 15 years, multi-implant PMMA embedded techniques have been frequently used with consistently good results reported. The main advantage of this method is its overall adaptability.

AA dorsal sutures, transarticular fixations and multi-implant PMMA cemented constructs constitute the 3 major reported methods of AA stabilization in dogs; however, other alternatives have been anecdotally reported in the literature. In 1984, Kishigami designed a complex dorsal tension band system allowing strong dorsal stabilization (Kishigami 1984). This system has not been subsequently reported in the literature except for a recent small case series (Pujol, Bouvy, et al. 2010). The use of bone plates was first reported by Thomas et al. in 1991 as a second line treatment when usual techniques had failed (Thomas, Sorjonen, et al. 1991). Since then, AA bone plating has infrequently been reported, although a recent small case series describes the use of a locking plate in toy breed dogs (Stead, Anderson, et al. 1993, Dickomeit, Alves, et al. 2011). A single case report of dorsal cross pinning technique was described in 1996 by Jeffrey, consisting of placing 2 pins from the axial spinous process to the wings of the atlas (Jeffery 1996). In 2014, Sanchez-Masian et al. described a new technique of dorsal suture stabilization using bilateral muscular sutures between the obliquus capitis caudalis muscle origin (on C2) and the contralateral obliquus capitis cranialis muscle insertion (on the occipital bone). This method was primarily designed to provide some degree of stabilization in dogs that are either too young (poorly ossified vertebrae) or too small (<1kg) to allow proper instrumentation (Sanchez-Masian, Lujan-Feliu-Pascual, et al. 2014). More recently, Beagle dogs have been used as a model for atlanto-odontoid joint replacement which constitute a brand new approach to AA instability attempting to restore its normal range of motion (Zang, Liu, et al. 2015).
Biomechanics of the surgically stabilized atlantoaxial joint

The biomechanical goal of most current stabilization techniques is to achieve rigid stabilization and facilitate arthrodesis of the joint. The rigidity is primarily assessed through evaluation of the remaining movement, expected to be minimal, and the stiffness value, expected to be high. By design, the dorsal suture systems are not rigid constructs and therefore cannot allow a similar fusion rate as the ventral rigid constructs. One study comparing the stiffness in ventral bending between the multi-implant PMMA embedded constructs and a dorsal technique (Kishigami tension band) revealed that the ventral technique was significantly more rigid (Kopf, Elder, et al. 2013). Unfortunately this is the only currently available study comparing the biomechanical properties of AA fusion techniques in dogs. It is therefore not known how different ventral stabilization techniques compare in terms of strength, stiffness or safety of implant placement. Furthermore, no study has compared the acute load to failure or the cyclic fatigue load of different techniques.

AA biomechanical data is very limited in dogs compared to the human counterparts in which multiple studies have compared the biomechanical characteristics of stabilization techniques (Grob, Crisco, et al. 1992, Hanley & Harvell 1992, Wilke, Fischer, et al. 1992a, Wilke, Fischer, et al. 1992b, Menezes & Traynelis 2008, Elliott, Tanweer, et al. 2012). The main surgical techniques used in humans are similar to those employed in dogs except that implants are placed dorsally. It has been demonstrated that the transarticular screw technique and internal fixation with multiple screws combined with rods offer the most rigid fixations (Menezes & Traynelis 2008). Dorsal suture techniques are indicated when a semi-rigid system is beneficial (pediatric medicine) or when anatomical anomalies do not allow pedicular screw placement. However, these semi-rigid techniques require concurrent external orthosis and are

**Optimal safe implantation corridors of the atlantoaxial joint and implantation accuracy**

In the AA region, most of the reported implant sites have been subjectively defined without detailed anatomical description. The only optimal safe implantation corridor that has been studied in dogs is the corridor used for transarticular fixation. Sorjonen and Shires provided a radiographic study determining ideal projected angles when they first developed the transarticular technique in 1981 (Sorjonen & Shires 1981). The main anatomical landmark used to define the corridor was the medial border of the alar notch; however an insertion point was not precisely defined. Based on measurements obtained in 18 mixed breed dogs, they described ideal projected angles of 29° (mediolaterally) and 21.5° (ventrodorsally) using the craniocaudal axis for reference.

A more recent publication using CT images in a group of toy breed dogs further described these same implantation corridors using slightly different definitions (Vizcaino Reves, Stahl, et al. 2013). The point of insertion was defined as “the deepest point of the pit medial to the cranial end of the crest running from the transverse process to the articular process”. The exit point of the corridor was defined as “the cranial opening of the transverse foramen of the atlas”. Using these definitions, the authors obtained slightly different projected angle values than Sorjonen and Shires (40 ± 1° mediolaterally and 20 ± 1° ventrodorsally). However, the authors did not clearly establish why they used these definitions ignoring the previously used alar notch. There was also minimal discussion on the fact that quality of vertebral alignment during CT imaging, which was not graded, could have significantly influenced their results given that reference points from 2 different vertebrae were used. Therefore, the reported values should be
interpreted with caution, and only applied using their precise definition of implantation corridors. Further investigation using a more traditional optimal safe implantation corridor would be necessary to determine which projected values should be used in practice. That same anatomical CT study also determined that the transarticular safe corridor width ranged from 3 to 4.5mm for dogs less than 5kg (Vizcaino Reves, Stahl, et al. 2013). Based on that result, those authors recommended using screws of 1.5-2mm diameter in toy breed dogs.

Other reported implantation sites, including the C1 lateral masses, C1 ventral arch, C2 cranial articular surfaces, C2 caudal vertebral body, C2 cranial vertebral body and C2 pedicles have been used without detailed anatomical description of the optimal safe corridors. One study reported a value of 50° (range: 45-60°) for the transverse projected angles of C2 pedicular screws as part of a CT spinal study involving dogs of various sizes (Watine, Cabassu, et al. 2006). There is currently no data available in veterinary medicine regarding the accuracy of implant placement in the AA region. Given the high rates of vertebral canal violation reported at other sites of the cervical spine, it can be suspected that similar incidents frequently occur in the AA region.

In humans, several studies described the morphometrics of bone corridors for AA implant insertion either on cadavers or CT images (Igarashi, Kikuchi, et al. 2003, Chung, Lee, et al. 2006, Chern, Chamoun, et al. 2009, Ji, Wang, et al. 2012). Unfortunately, it is impossible to apply the data obtained in humans to the canine population as vertebral conformation is very different. Actually, the human atlas and axis have well developed lateral masses which allow lateral implant placement from a dorsal approach. This is certainly the main reason why a dorsal approach is most commonly performed in humans whereas a ventral approach is more commonly preferred in dogs (Menezes & Traynelis 2008).
Success rates and limitations

The most severe and dreaded complication associated with AA stabilization techniques is iatrogenic acute spinal cord injury due to intraoperative vertebral manipulation or spinal cord contusion/laceration/compression associated with inappropriate implant placement. Such an event can result in immediate or postoperative cardiorespiratory arrest and death. The immediate mortality rates associated with surgical procedures have been estimated in one retrospective study to be approximately 5% for ventral stabilization techniques and 8% for dorsal techniques (Plessas & Volk 2014). Because of the higher mortality rates compared to most spinal surgeries, AA stabilization remains one of the most challenging neurosurgical procedures performed in veterinary medicine.

Another common complication is implant failure and loss of stabilization and has been reported in 25% for dorsal techniques and 18% for ventral techniques (Sanders, Bagley, et al. 2004). This can occur prior to AA fusion which can result in relapse in subluxation and neurological deficits. If implant failure occurs after significant arthrodesis is achieved, the failure may remain asymptomatic. However implant migration can also occur and cause draining tracts or central nervous system injury (Johnson & Hulse 1989, Thomas, Sorjonen, et al. 1991, Wheeler 1992, Schulz, Waldron, et al. 1997, Beaver, Ellison, et al. 2000, Sanders, Bagley, et al. 2000). Some authors have anecdotally suggested that the multi-implant technique may offer a lower rate of implant failure (Sharp & Wheeler 2005). Other reported complications have included tracheal pressure necrosis, esophageal stricture, laryngeal paralysis and pneumonia (Thomas, Sorjonen, et al. 1991, McCarthy, Lewis, et al. 1995, Sanders, Bagley, et al. 2000).

It should be emphasized that the data available in the literature is very limited and that comparison between methods are difficult to interpret due to the subjective definition
of success rates and poor definition of causes of mortality. If we exclude studies that have 5 cases or less, to limit potential selective bias, a few trends can be observed (Table II.1). A successful outcome with dorsal suture is obtained in approximately 60% of the cases (range 29-75%) while mortality rate is around 25% (range 8-39%) (Chambers, Betts, et al. 1977, Denny, Gibbs, et al. 1988, Thomas, Sorjonen, et al. 1991, Beaver, Ellison, et al. 2000). In comparison, transarticular fixation achieves a success rate of 71% (range 44-90%) with a similar mortality of 22% (range 10-44.4%) (Denny, Gibbs, et al. 1988, Thomas, Sorjonen, et al. 1991, Beaver, Ellison, et al. 2000, Jeserevics, Srenk, et al. 2008). It should be noted that one study by Thomas et al. in 1991 obtained particularly poor results for both techniques compared to any other publications and therefore may not be representative of the typical population. If we exclude that particular study, dorsal suture method reaches almost a 67% success rate but maintains a poor mortality rate of 24%, and the success rate for transarticular fixation becomes 78% with a 15% mortality rate. In both situations, transarticular fixation appears to offer superior results than dorsal suture. A similar analysis conducted for multi-implant PMMA cemented constructs suggests a success rate of 87% and a mortality rate of 10%, seemingly superior to the two previous techniques (Schulz, Waldron, et al. 1997, Knipe, Sturges, et al. 2002, Platt, Chambers, et al. 2004, Sanders, Bagley, et al. 2004, Beckmann, Mazzanti, et al. 2010, Aikawa, Shibata, et al. 2013).

One study identified risk factors correlated to postoperative outcome where dogs younger than 2 years old, presenting clinical signs for less than 10 months or suffering from milder neurological deficits had a significantly better outcome after surgical correction (Beaver, Ellison, et al. 2000).

Again, these results should be interpreted with caution because of multiple potential confounding factors, such as the cause of mortality or the time of publication. Actually, it
is noteworthy that the 3 main methods of stabilization have been used successfully over time with the dorsal suture mostly reported in the 1980’s, the transarticular fixation in the 1990’s and the multi-implants PMMA cemented constructs mostly after 2000. Nevertheless, the fact that several studies have suggested that cemented constructs can achieve satisfactory success rates is a significant piece of information for veterinary clinicians and neurosurgeons. Overall, ventral surgical stabilization likely provides a better outcome than conservative management even though prospective studies would be necessary to ascertain that statement (Havig, Cornell, et al. 2005).

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<td>75</td>
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<td>Aikawa 2011</td>
<td>49</td>
<td>47</td>
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<td>Overall</td>
<td>40</td>
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Table I.1  **Summary of selected AA instability case series success rates and mortality rates.**

N: number of cases; Ambul Pre-Tx: percentage of cases ambulatory prior to surgery; Success: percentage of successful cases as defined in cited reference; Death Post-Tx: percentage of dogs that died within the follow-up period; n/a: data non-available.
VII. Conclusion

Upon review of the available literature on canine AA instability, it is clear that many aspects of the disease are still poorly understood. The limited physiological and anatomical descriptions of the AA joint have emphasized that the odontoid process is a cornerstone of the AA motion unit stability. This has lead clinicians to focus on establishing evidence of failure of the atlanto-odontoid system to diagnose AA instability. However, there is no well-established gold standard to diagnose this disease and therefore sensitivity and specificity of current imaging modalities are unknown. In that context, performing dynamic studies to further demonstrate instability is particularly controversial. Safer methods such as conspicuous MRI studies including ligamentous structure assessment may reveal to be a superior diagnostic tool. Furthermore, the prevalence of asymptomatic AA instability associated with incidental odontoid anomalies is unknown. If that prevalence is high in the population of toy breed dogs, it could significantly decrease the specificity of any imaging modality focusing on dynamic assessment.

Even when those diagnostic difficulties can be overcome, the decision making that follows diagnosis remains extremely challenging due to limited data available. The simplest approach would be to consider that evidence of instability is sufficient to justify surgical stabilization. This logical approach has been advocated in human medicine; however it has been supported by an excellent surgical outcome achieved with modern techniques. If veterinary neurosurgeons were able to provide similar results, such a proactive paradigm would likely be equally justified in canine patients. Unfortunately, the mortality risk associated with current stabilization techniques (5-25%) has often prompted clinicians to adopt a more conservative approach. Medical management seems to be most successful in dogs having presented with deficits for less than a
month. However, surgical outcome is superior in dogs with milder deficits.
Unquestionably, a consensus on the minimal criteria that should be observed to recommend stabilization in dogs is still lacking. In contrast, the demonstration of instability with concurrent neurological deficits is often sufficient to recommend surgical treatment in humans. Based on that, most canine cases of AA instability are potential surgical candidates.

Three major techniques have been used to stabilize the AA joint (dorsal suture, transarticular fixation and multi-implants PMMA embedded construct). Multiple variations of these techniques are described, including multiple subjectively defined screw/pin implantation sites, but the optimal safe implantation corridors of atlas and axis have not been extensively studied. Experimentally, little work has been conducted to provide rigorous comparisons. Dorsal suture stabilization has been established to have lower stiffness than multi-implant constructs, but biomechanical properties of ventral techniques have not been compared. Similarly, technical outcomes (such as implant placement accuracy) have not been determined or compared between techniques. Based on the limited clinical data available, the multi-implant PMMA embedded constructs seems to consistently achieve good results and may represent the most reliable method, however, proper clinical comparative studies are lacking. Overall, the choice of stabilization technique remains based on the surgeon’s preference and level of comfort, however, the type of instability, anatomical morphology, bone quality, and growth stage of the patient should also be taken into consideration.
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CHAPTER II - DESCRIPTION OF THE OPTIMAL SAFE IMPLANTATION CORRIDORS FOR VENTRAL ATLANTOAXIAL STABILIZATION USING OSIRIX™

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I. Introduction


Complications reported in the literature include perioperative and intraoperative spinal cord and/or brainstem injury, implant failure, implant migration, tracheal pressure necrosis, esophageal stricture, laryngeal paralysis and pneumonia (Thomas, Sorjonen, et al. 1991, McCarthy, Lewis, et al. 1995, Sanders, Bagley, et al. 2000). Iatrogenic mortality is certainly the most dreaded complication and various causes can be suspected during AA stabilization. Excessive manipulation of the head or AA joint can easily aggravate an already existing AA subluxation and cause unrepairable damage to the spinal cord. Violation of the vertebral canal (VC) by the stabilizing implants represents another source of iatrogenic spinal cord injury. The incidence of VC violation associated with AA stabilization is unknown in dogs but it is likely underestimated by clinicians given the low sensitivity of postoperative radiographs (Hettlich, Fosgate, et al. 2010). Experimental studies on ventral placement of pedicular and monocortical implants in the cervical spine have demonstrated that VC violation is common and it is likely that similar violation occurs in AA stabilization (Hicks, Pitts, et al. 2009, Hettlich,

Ventral AA stabilization techniques offer multiple implant placement options. This has resulted into a substantial variation of implant positioning described in the literature with often little to no objective data to support it. Traditionally, vertebral optimal safe implantation corridors (OSICs) have been characterized using computed tomography (CT) images. This has been achieved by identifying a theoretical plane within which the optimal implant is to be positioned. Using such a predefined plane allows simplification of a complex tridimensional (3D) problem into a bidimensional (2D) description. This method has been applied successfully along most of the vertebral column because stabilizing implants are typically positioned within the transverse plane of the vertebra (Watine, Cabassu, et al. 2006, Corlazzoli 2008, Knell, Kircher, et al. 2011, Vallefuoco, Bedu, et al. 2013). However, most of the reported atlas (C1) and axis (C2) implantation sites have an oblique direction which precludes the use of traditional methods of OSIC description. As a result, AA implant sites have been subjectively defined without detailed anatomical description. The only OSIC that has been studied in dogs is the corridor used for TSF fixation (Sorjonen & Shires 1981, Vizcaino Reves, Stahl, et al. 2013). However, available studies have used different subjective definitions of the optimal implant position.
and therefore obtained slightly different results.

New methods of optimal implant placement determination are needed to generate objective definitions of complex oblique implantation sites. OsiriX™ (Pixmeo SARL, Bernex, Switzerland) is an open-source Digital Imaging and Communication in Medicine (DICOM) software used in human medicine for 3D preoperative planning and intraoperative guidance (Mandel, Amorim, et al. 2013, Bizzotto, Costanzo, et al. 2014). The accuracy and reliability of the software have been validated experimentally and compared to other image analysis programs (Yamauchi, Yamazaki, et al. 2010, Fortin & Battie 2012, Kim, Jung, et al. 2012). Good accuracy and low measurement error of the software have been demonstrated which is critical for surgical planning of implant placement with narrow safety margins.

In order to study the commonly used ventral AA OSICs, we developed a novel bone corridor analysis method allowing objective description of complex implantation sites using OsiriX™. The purposes of the present study were: 1- to describe the CT based method of implant simulation, 2- to validate the method and estimate its accuracy, 3- to use this method to describe ventral OSICs of the AA region in dogs, 4- to compare AA OSICs of dogs suffering from AA instability (AAI) with non-affected dogs. We hypothesized that OsiriX™ would provide accurate description of ventral AA implant placements with acceptable measurement error relative to the available safety margins.
II. Materials and methods

Preliminary review of the literature

An online literature search was conducted prior to the study in order to identify available descriptions of canine AA anatomy as well as bone corridors previously used for ventral AA stabilization. Both PubMed and Google scholar search engines were used to identify pertinent publications with the search terms “ventral atlantoaxial dog”, “atlantoaxial instability dog” or “atlantoaxial subluxation dog”. When using Google scholar, only the first 250 references were assessed for relevance. The articles written in another language than English were excluded. All identified journal articles and textbook chapters describing AA anatomy or ventral AA stabilization techniques were reviewed in detail, including identification of pertinent cited references which were also reviewed.

Computed tomography image acquisition and analysis

Between October 2012 and December 2013, dogs were prospectively recruited in order to obtain a CT scan of their craniocervical region. The initial objective was to recruit approximately 10 dogs affected with AAI and 20 dogs with a normal AA joint (10 Toy breed + 10 Beagle dogs). A minimal age of 6 months was subjectively selected to avoid excessive anatomical variations due to growth stage. Toy breed dogs were defined as dogs with a body weight less than 5kg. The CT scans obtained for dogs with a normal AA joint were either obtained from cadavers or client owned animals anesthetized at the Ontario Veterinary College Health Science Centre (OVC HSC) for clinical reasons unrelated to this study. Owner consent was obtained for all client owned dogs. For AAI dogs, obtaining a CT scan of the AA region was part of the OVC HSC standard practice for diagnostic workup and/or pre-surgical planning. A diagnosis of AAI
was reached if dens separation, agenesis or hypoplasia was identified with clinical signs consistent with a cranial cervical myelopathy or if unequivocal AA subluxation was visible in the CT study.

CT images of the craniocervical junction were obtained using a 16 slice detector GE Brightspeed CT scanner (GE Healthcare, Milwaukee, WI, USA). The raw data was acquired with a standardized protocol in helical mode at 25cm collimation, 1.0s rotation time, 0.562:1 pitch, 120kV and 250mAs using both standard and bone algorithms. Both algorithms were reviewed but only the images captured in a bone algorithm were used for OSICs analysis.

The images were subsequently imported into the free open-source software version of OsiriX™ DICOM viewer (OsiriX™ versions 5.8-6.0) using an Apple® computer (Mac mini, Apple Inc., Cupertino, CA, USA). The images were reviewed using the window width and level preset in OsiriX™ for bone CT images. The images were analyzed using the 2D viewer, 3D multi-planar reconstruction (MPR) and 3D volume rendering (VR) modes.

**3D simulation and 3D position of optimal implant placement using OsiriX™**

In order to define objective optimal implant placements for each available bone corridor, we developed a method based on geometrical simplification of the bone corridor. The purpose of this simplification was to obtain 3D geometrical shapes with well-defined centered axes. Optimal implant placements were then defined as axes centered within the simplified bone corridor. To be surgically applicable, the insertion point of such implant placement also had to be located on the ventral surface of C1 or C2. The geometrical shapes simulating the bone corridors were delineated in OsiriX™ by placing region of interest (ROI) points either in 3D-MPR mode or 3D-VR mode. These
ROI points were subsequently used as landmarks in 3D-MPR mode to determine centered axes of the bone corridors using various geometrical methods (Fig II.1, Appendix I). Each optimal implant placement could then be simulated by placing 2 ROI points along the centered axis representing the insertion and exit points of the implant.

![Fig II.1 Principles of determination of optimal implant placement using the geometrically centered method.](image)

(A) Example of simplification of a bone corridor into a pyramidal shape using ROI points placed in 3D-VR mode.
(B) A centered axis can subsequently be determined in 3D-MPR mode using ROI points as landmarks (see Appendix I for details).

Once the optimal implant placement was simulated, its 3D position was defined objectively using landmarks available to the neurosurgeon. This was achieved by defining the insertion point using anatomical landmarks while the direction of the implant was defined by projecting the implant onto 2 anatomical planes (sagittal, dorsal or transverse).

The anatomical landmarks used to define the insertion points are summarized in Fig II.2.A. These landmarks were positioned on a screen copy of the ventral view of the AA joint with the insertion ROI points using CorelDRAW® drawing software (CorelDRAW® Graphic Suite version X7, Corel Corp., Ottawa, ON, Canada). In some cases, 2 different
screen copies apposed together were used for C1 and C2 to correct for significant misalignment of the 2 bones \textit{i.e.} AA joint scanned in a non-neutral position). A standardized diagram was then used to visually reproduce the relationships between the landmarks and the insertion points (Fig II.2.B). The diagrams of all dogs were eventually fused together to produce an image representing the distribution of the insertion points with respect to the landmarks within the sampled population.

\textit{Fig II.2} \textbf{Principles of determination of optimal insertion points using OsiriX™ and CorelDRAW®.}

(A) Landmarks used to define the insertion points of each optimal implant on a CT image including a sagittal line (Sag), the lateral border of C2 (Lat), the cranial border of C1 caudal articular surface (Cr), the caudal border of C2 cranial articular surface (0°), oblique lines rotating around the center of the 0° line (30° and 60°), the caudal border of C2 vertebral body (Cd) and a diagonal line passing through the center of the 0° line and the intersection between Lat and Cd. Distances between parallel lines were further divided into thirds.

(B) Insertion points placed on a standardized AA diagram defining their position with respect to the landmarks.

The implant direction was defined using 2 projected angles (ProjA) on anatomical planes, which were used as 3D coordinates (Fig II.3.A). It is important to note that projections onto anatomical planes are not identical between C1 and C2 because of the
significant range of motion existing between these vertebrae. In this study, a projection on C1 anatomical planes was operated for C1 implants and respectively for C2 implants. Transarticular implants involving C1 and C2 were defined using C1 projections. OsiriX™ allowed determination of the ProjA by aligning 3D-MPR planes with anatomical planes. This was achieved by identifying 1 anatomical plane and 1 anatomical axis. For both C1 and C2, the sagittal plane was defined as the plane of symmetry of the vertebra which was determined in 3D-MPR mode. Then, an anatomical axis was identified in the sagittal plane to complete the alignment of all 3 planes. For C1, the ventrodorsal axis was defined as the sagittal cranial border of C1 dorsal and ventral arches. For C2, the craniocaudal axis was defined as the sagittal ventral border of the C2 vertebral foramen (Fig II.3.B). Once anatomical alignment was obtained in 3D-MPR mode, ProjA could be measured by centering the intersection of the 3 planes on the insertion point followed by shifting the plane of interest until the exit point was visualized (Fig II.3.C).

Fig II.3  Manual measurement of ProjA using OsiriX™.
(A) Sagittal, transverse and dorsal ProjA can be used as the implant 3D coordinates.
(B) To determine ProjA in using OsiriX™ 3D-MPR mode, the 3 planes are first aligned with the anatomical planes (points OAB are used as landmarks – see Fig II.5).
(C) ProjA measurement is obtained by shifting the plane of projection from the insertion point (I) to the exit point (E) of the studied implant (example of dorsal ProjA).
Estimation of the safety margins of each OSIC

In order to provide an estimation of the safety margins associated with each implant site, we developed a new method considering both the bone margins and the diameter of the implant used. The general principle of the method was to study the bone corridor associated with each optimal implant site in 2 subjectively defined orthogonal planes using OsiriX™ 3D-MPR mode. In each of these planes, 2 safety margins were determined by rotating the central axis of the implant away from the optimal position around the insertion point until the implant position became considered unsafe (either due to inappropriate bone purchase or violation of vital structures). The first point encountered along each rotation of the implant that was causing it to become unsafe was identified by placing an ROI point representing a safety margin of the bone corridor. For sagittal implants directed toward the vertebral canal, 75% of the corridor length was used to position safety margin points. This method allowed identification of 4 safety margin points for each bone corridor. Safety angles (SafA) could then be determined using the optimal implant ROI points, the safety margin ROI points and circles simulating the implant diameter as demonstrated in Fig II.4.

First, 2 orthogonal planes containing the safety margin points are identified (green points). The intersection of these 2 planes simulates the implant site (IE). Then, circles of the same diameter as the simulated implant are positioned around the safety point (green circles). A tangent line to each circle passing through the insertion point is used to measure SafA (red arrows).

Fig II.4  Manual method of determination of SafA using OsiriX™ 3D-MPR mode.
Mathematical model and semi-automated method for OSiC analysis

The previously described methods of determination of ProjA and SafA were considered excessively time consuming to apply on a large sample size. Given that we intended to study multiple implant sites in approximately 30 dogs, we elected to develop a method of mathematical calculation of these angles that would be more time efficient. All mathematical calculations were performed using Microsoft® Excel software (version 2011, Microsoft Corp., Redmond, WA, USA). The calculations were based on the CT 3D coordinates of the ROI points simulating the optimal implants and their respective safety margins. An open-source OsiriX™ plugin (ExportROIs version 1.3.1) allowing fast exportation of 3D coordinates into Microsoft® Excel was used to optimize the process and limit the risk of error while transferring the 3D data.

Mathematical determination of ProjA required advanced vectorial calculations that are detailed in Appendix II. Briefly, each optimal implant was considered as a vector oriented from its insertion to exit points. The coordinates of these points provided by the CT scan could not be directly utilized for ProjA calculations. Instead, a change of coordinate system (also called change of basis) was performed to provide the vector coordinates with respect to the anatomical axes. This was achieved by defining anatomical coordinate systems for C1 and C2 using 3 ROI points strategically selected in the sagittal plane of each vertebra (Fig II.5.A). Once the coordinates of the vector were determined with respect to the anatomical axes, the ProjA could be easily calculated using standard trigonometric equations (Fig II.5.B). Each step of the calculations was entered into an excel sheet as depicted in Appendix III.

Mathematical equations for calculation of the SafA could be established through trigonometry as depicted in Fig II.6.A. The CT coordinates of the safety margins ROI points could be used without any change of basis, as these angles are defined with
respect to the axis of the optimal implant. The diameter of the implant used had to be known to calculate SafA. The same safety margin ROI points could also be used to estimate the width of the bone corridor in both orthogonal planes as depicted in Fig II.6.B.

Fig II.5  Geometrical demonstration of ProjA calculations based on anatomical coordinates (after change of basis has been operated).

(A) Anatomical coordinate systems were defined for C1 and C2 by positioning 3 points (O, A, B) in the sagittal plane of each vertebra. The point O represents the origin of the coordinate system, while (AB) represents the craniocaudal axis (x).

(B) Geometrical demonstration of sagittal (yellow), dorsal (blue) and transverse (purple) ProjA ($\theta$) calculation based on anatomical coordinates ($x$, $y$, $z$) of a vector ($\vec{IE}$).

Fig II.6  Geometrical demonstration of SafA and OSIC widths calculations.

(A) Method of SafA calculation:

$$SafA = \alpha - \beta$$

$$\alpha = \cos^{-1}(\frac{\vec{IE} \cdot \vec{IS}}{||\vec{IE}|| \cdot ||\vec{IS}||})$$

$$\beta = \sin^{-1}(\frac{r}{||\vec{IS}||})$$

(B) Method of OSIC width calculation:

$$Wd = ||\vec{IS}|| \cdot \sin(\alpha)$$

I: Insertion point, E: Exit point, S: Safety margin point, r: Radius of the implant.
Mathematical model validation and estimation of angle measurement errors

In order to validate our semi-automated method of determination of ProjA and SafA, a complete OSICs simulation was performed by 1 author (GL) including simulation of optimal implants (2 ROIs/implant), safety margins (4 ROIs/implant) and C1/C2 anatomical axes (6 ROIs/dog) in all recruited dogs. A sample of 12 dogs was then randomly selected within the recruited population and 1 implant was randomly selected for each dog (see Appendix IV). Prior to any angle measurements and calculation, the ROI points simulating the selected optimal implant and its associated anatomical coordinate system and safety margins were imported in OsiriX™ for each dog. These ROI points were then used to determine 2 ProjA and 4 SafA mathematically and compared to measures obtained manually for each of the 12 implants. The manual measures were obtained by 2 observers (AZ and GL) and repeated after a 1 week interval. For determination of SafA, implant diameter was subjectively set at 1.5mm for this part of the study. Agreements between the calculated values and manual measures and 95% tolerance limit intervals were determined to validate the mathematical model.

In order to estimate the error generated by the operator when measuring ProjA and SafA manually, calculated values were considered as gold standard. The measurement error was determined for each observer by calculating the absolute difference between the manually measured and calculated values.

In order to estimate the error on ProjA measurements generated by the operator when identifying the anatomical axes, 6 ROI points representing the anatomical coordinate systems of C1 and C2 were positioned by 2 observers (AZ and GL) with 2 repeats in all 12 dogs. This provided 4 sets of 6 ROIs per dog, each representing the same anatomical axes with a slight variation due to operator variability. To estimate the effect of this variability on ProjA values, all previously determined ROI points
representing all of the studied implant sites were imported into OsiriX™ for all 12 dogs. For each implant site, 2 ProjA were calculated 4 times based on the 4 coordinate systems obtained by the 2 observers. The mean of the 4 repeats of each ProjA values was used as gold standard for that part of the study, because the exact position of the anatomical axes cannot be objectively determined. Agreements and absolute error between each of the 4 obtained values and the gold standard as well as 95% tolerance limit intervals were determined to estimate the ProjA calculation error. Agreements and 95% tolerance limit intervals were also determined between observers, between repeats and within subsamples (specific coordinate systems or specific projection planes) in order to identify the most significant sources of error.

**Application of the semi-automated method for analysis of AA OSICs**

Once the mathematical model was validated, the optimal implant 3D positions and associated safety margins were determined in all recruited dogs for each AA bone corridor described in the literature. The described mathematical method was used to calculate the optimal implant ProjA, SafA and bone corridor widths. For bilateral implants, right and left sides were considered as the same implantation site in the data analysis. As both sides have opposite directions with respect to the sagittal plane, subjective conventions were defined for orientation of ProjA to obtain values with identical sign regardless of the side. These conventions were specified when reporting the results to allow proper orientation in space. The SafA were determined using simulated implants of 1.5, 2 and 2.4mm diameters.

The main purpose of this part of the study was to provide an objective description of the OSICs and to compare their characteristics between AAI dogs, non-affected Toy breed (N-Toy) dogs and non-affected Beagle (N-Beagle) dogs. The diameter of implants
used for intergroup comparison of SafA was 1.5mm in AAI and N-Toy dogs which were compared to 1.5, 2 and 2.4mm diameters in N-Beagle dogs. Critical angles were defined to optimize the clinical significance of the statistical comparisons of SafA. A critical safety angle (CritSafA) was defined as the smallest of the safety angles that also represented a safety margin for VC violation. A technical safety angle was defined as the sum of 2 SafA determined within the same plane. A critical technical angle (CritTecA) was defined as the smaller of the 2 technical safety angles. In other words, CritSafA estimated the angular margin for error preventing vertebral canal violation while CritTecA estimated the minimal angular width of the bone corridor. For each OSICs, ProjA, CritSafA, CritTecA, and widths were statistically compared between groups.

**Statistical Analysis**

Agreements between different methods of angle determination (automatically calculated vs. manual) and between repeated measures were obtained using the Bland-Altman method and concordance correlation. This method also allowed calculation of 95% tolerance intervals, representing the range of values that would theoretically be obtained for a single measure with 95% probability. These values estimated the reproducibility of the method which is defined as the degree to which repeated measurements provide similar results (de Vet, Terwee, et al. 2006).

The distribution of continuous dataset was tested for normality prior to statistical testing. Normality was evaluated using a Shapiro-Wilk test and examination of the residuals. If the data was normally distributed, a general linear mixed model was used to compare the groups. If the data was not normally distributed, a Kruskal Wallis test (non-parametric one-way ANOVA) was used instead. Categorical variables were analyzed using Chi-Square test. Statistical analysis of the data was performed using statistical

Statistical significance was set at 0.05.
III. Results

Sampled population and CT images acquisition

Over the recruitment period, 27 dogs were recruited to participate in this study. Table II.1 summarizes the signalment, neurological deficits, reason for hospitalization and main findings on CT images of the AA region for each recruited dog. The recruited population differed slightly from the initial objective including 5 AAI dogs, 13 N-Toy dogs and 9 N-Beagle cadavers. The weight (mean±SD) of AAI dogs was 2.32±1.09Kg which was statistically different from N-Toy dogs: 3.32±1.15Kg (p= 0.0121) and N-Beagle dogs: 8.44±0.92Kg (p<0.0001). The median age was 27 months for AAI dogs and 67 months for N-Toys (p=0.0545). The age of Beagle cadavers was unavailable but they were of adult size with no visible growth plates on CT. One N-Toy dog (case 7) was 3 weeks younger than our initial subjective inclusion criteria. However, the CT images revealed complete fusion of the vertebral growth plate suggesting the AA region had reached adult stage. The overall ratio male/female was 0.48 and was not statistically different between groups (p=0.3800).

CT images of the craniocervical junction were successfully obtained in 25 dogs using the pre-established protocol. In 2 AAI dogs (case 1 and 4), the slice thickness was set at 1.25mm instead of 0.625mm due to a protocol error. It was elected to include these 2 cases in the study despite this error, due to low number of recruited AAI dogs.

Table II.1  Signalment, neurological deficits, reason for hospitalization and pertinent CT findings of the craniocervical junction within the sampled population (see next page)
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<th>Sex</th>
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<th>Main neurological deficits</th>
<th>Reason for general anesthesia / euthanasia</th>
<th>CT findings (craniocervical junction)</th>
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<td>Dull mentation (non-ambulatory) Cardiorespiratory arrests (partial neurological examination)</td>
<td>AA instability Metabolic epilepsy (hypoglycemia)</td>
<td>Dens agenesis / AA subluxation (↑ dorsal AA distance) Caudal occipital hypoplasia</td>
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**AAI dogs**
**Non-affected Toy breeds**
**Non-affected Beagles**

Red font: Subpopulation selected for validation study

*: CT slice thickness of 1.25mm

1. CT performed on cadavers
AA OSICs and determination of optimal implant placement 3D positions


Each corridor was simplified into a geometrical shape, including pyramids, prisms and hemi-ellipsoids (Fig II.7). The general principle used to simulate optimal implants was to identify well-defined centered axes of the geometrical shapes (Appendix I). In addition, a transarticular optimal implant position was defined (C1-C2, bilateral) using the lateral mass corridors to define its axis and the ventral surface of C2 to define its insertion point. For the caudal vertebral body corridor, 3 different centered implant positions could be defined. Therefore in total, 13 optimal implant sites could be objectively defined (Fig II.7). For implants located in the sagittal plane, a traditional...
method was used by identifying 1 point of the optimal axis within the sagittal plane which was then used in 3D-MPR mode to center the implant axis (Appendix I.7).

Fig II.7  Simulation of 13 optimal implant placement using geometrical simplification of the commonly used AA bone corridors.

Overall the method was used successfully in all 27 cases. Some limitations were found during positioning of ROI points. Even though OsiriX™ 3D modes generated continuous 3D space, ROI points placed in 3D-MPR or 3D-VR modes remained associated to specific slices. In other words, the space located between each slice could not be represented using ROI points. Another seemingly random difficulty encountered in 3D-VR mode was an occasional software glitch when placing ROI points. Instead of positioning the point on the visible bone surface, the point would be placed on the opposite side of the vertebra. This malfunction could be overcome by positioning the vertebra so that the bone surface of interest was tangent to the operator view.
Validation of the mathematical model and estimation of measurement errors

The raw data of manually measured and calculated values from the 2 observers is presented in Table II.2.

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Table II.2 Mathematically calculated and manually measured values of ProjA and SafA.

Implants were numbered as follows: 0-1= C1 pedicular; 2-3= C1-C2 transarticular; 4-5= C2 cranial articular surface; 6-7= C2 pedicular; 8-9= C2 caudal vertebral body (parasagittal); Even # = Right side; Odd # = Left side.
The 2 CT studies that had 1.25mm slice thickness (instead of 0.625mm) were excluded of the random sampling process. Sagittal implants were also excluded due to only 1 projected angle value defining them. There was excellent agreement between the calculated and measured values for both ProjA ($\rho_c=0.9986$) and SafA ($\rho_c=0.9996$) (Fig II.8).

The 95% tolerance interval obtained by concordance analysis to estimate operator-induced error for manual angle measurements by comparison to semi-automated calculations (gold standard) were respectively, [-1.23°,1.20°] and [-0.65°,0.70°] for ProjA and SafA. Absolute errors were, respectively, [ProjA= 0.44 ±0.53°; SafA= 0.27±0.25°] and [ProjA= 0.26±0.21°; SafA= 0.18±0.18°], for each observer (Fig II.8). Manual determination of angles was therefore considered a reproducible method with minimal operator-induced error. Absolute error and concordance data is presented in Table II.3.

<table>
<thead>
<tr>
<th>Angle</th>
<th>Meas. 1</th>
<th>Meas. 2</th>
<th>n</th>
<th>$R^2$</th>
<th>$\rho_c$</th>
<th>Low TL</th>
<th>Up TL</th>
<th>Low CI</th>
<th>Up CI</th>
<th>Bias</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ProjA</td>
<td>Math</td>
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<td>0.9972</td>
<td>0.99859</td>
<td>-1.23</td>
<td>1.20</td>
<td>-0.13</td>
<td>0.09</td>
<td>-0.0194</td>
<td>0.7264</td>
</tr>
<tr>
<td>Rep1</td>
<td>Rep2</td>
<td></td>
<td>48</td>
<td>0.9944</td>
<td>0.99714</td>
<td>-1.94</td>
<td>1.75</td>
<td>-0.32</td>
<td>0.13</td>
<td>-0.0917</td>
<td>0.4146</td>
</tr>
<tr>
<td>Obs1</td>
<td>Obs2</td>
<td></td>
<td>48</td>
<td>0.9937</td>
<td>0.99683</td>
<td>-1.99</td>
<td>1.92</td>
<td>-0.27</td>
<td>0.20</td>
<td>-0.0333</td>
<td>0.7787</td>
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<tr>
<td>SafA</td>
<td>Math</td>
<td>Single value</td>
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<td>0.9992</td>
<td>0.99961</td>
<td>-0.65</td>
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<td>0.07</td>
<td>0.0268</td>
<td>0.2390</td>
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<tr>
<td>Rep1</td>
<td>Rep2</td>
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<td>0.9984</td>
<td>0.99918</td>
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<td>1.00</td>
<td>-0.11</td>
<td>0.07</td>
<td>-0.0195</td>
<td>0.6774</td>
</tr>
<tr>
<td>Obs1</td>
<td>Obs2</td>
<td></td>
<td>96</td>
<td>0.9983</td>
<td>0.99907</td>
<td>-0.94</td>
<td>1.17</td>
<td>0.02</td>
<td>0.21</td>
<td>0.1155</td>
<td>0.0186</td>
</tr>
</tbody>
</table>

Table II.3 Manual measurement concordance analyses and absolute error calculations. $n$: number of measures compared, $R^2$: correlation coefficient, $\rho_c$: concordance coefficient, TL: 95% tolerance interval limit (degrees), CI: 95% confidence interval limits (degrees), Obs: observer, Rep: repeat.

Cells in green highlight the most relevant results, Cells in orange highlight statistically significant bias (which was not considered clinically significant).
Fig II.8  Reproducibility and absolute error caused by manual angle measurements (Bland-Altman plots and box plots of the 4 quartiles).

Bland-Altman plots are used to represent the difference between each measurement and the gold standard (GS) on the y axis. With perfect agreement between the 2 compared methods, all the points would be located on the 0 line. The 2 lines parallel to the centre line represent the 95% tolerance limits which is the expected error on a single measurement with respect to the gold standard with 95% probability.

(A) Agreement and absolute error of manual ProjA compared to calculated values.
(B) Agreement and absolute error of manual SafA compared to calculated values.

On estimation of operator-induced error on calculated ProjA values when placing the landmarks of the anatomical axes (OAB points), agreements between each values and the mean of 4 values was excellent ($\rho_c=0.9985$) with an overall low 95% tolerance interval [-1.62°, 1.61°] (Fig II.9). The absolute error (mean±SD) was determined for each individual value (0.58±0.54°), the mean of 2 values from the same observer (0.42±0.39°) and the mean of 2 values from different observers (0.30±0.25°). Agreement analysis within subsamples considering the coordinate system used (C1 or C2) and the plane of projection (Sagittal / Transverse / Dorsal) conducted between observers and between
repeats revealed the widest 95% tolerance interval was obtained when comparing inter-
observer values of ProjA calculated in C1 coordinate system [-3.58°, 3.72°] (Table II.4).
These results suggest that the simulation of anatomical axes in OsiriX™ using the 
previously defined OAB points is very reproducible. The largest obtained error on an 
individual measurement was estimated at 3.7° (with 95% probability).

<table>
<thead>
<tr>
<th>Concordance analysis used to estimate reproducibility of OAB simulation and estimate error due to OAB positioning</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angle</td>
</tr>
<tr>
<td>Mean 4 values</td>
</tr>
<tr>
<td>Mean 4 values</td>
</tr>
<tr>
<td>Obs 1-Sag</td>
</tr>
<tr>
<td>Obs 1-Tr</td>
</tr>
<tr>
<td>Obs 1-Dors</td>
</tr>
<tr>
<td>Obs 1-C1</td>
</tr>
<tr>
<td>Obs 1-C2</td>
</tr>
<tr>
<td>Mean 4 values</td>
</tr>
<tr>
<td>Rep 1</td>
</tr>
<tr>
<td>Rep 1-Sag</td>
</tr>
<tr>
<td>Rep 1-Tr</td>
</tr>
<tr>
<td>Rep 1-Dors</td>
</tr>
<tr>
<td>Rep 1-C1</td>
</tr>
<tr>
<td>Rep 1-C2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Absolute ProjA error due to OAB (compared to mean of 4 values)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rep 1</td>
</tr>
<tr>
<td>Obs 1</td>
</tr>
<tr>
<td>Obs 2</td>
</tr>
<tr>
<td>Mean 2 Obs</td>
</tr>
</tbody>
</table>

Table II.4  Anatomical simulation (OAB point placement) concordance analyses and absolute error calculations.


Cells in green highlight the overall as well as intra/inter-observer reproducibility and measurement errors, Cells in red highlight subsample analyses with wider tolerance limits, Cells in orange highlight statistically significant bias (which were not considered clinically significant).
Description of the insertion points with respect to ventral anatomical landmarks

The optimal implant insertion points could be positioned on the standardized diagram in all cases and summarized by drawing insertion areas for each implant site (Fig II.10). The overall variation between groups of the optimal insertion points with respect to the landmarks was considered small. The only exception was a N-Toy dog (case 7), for which some insertion points were slightly separated from the rest of the sampled population (see Fig II.10). Interestingly, this dog was also the youngest and had the smallest body weight of the sampled population. Based on this diagram, objective definitions of insertion points were generated (Table II.5).

OSICs characteristics in affected and non-affected dogs

OSICs characteristics including ProjA, widths, lengths and SafA could be calculated in all dogs for all implantation sites. Each ProjA angle value was expressed with respect to the anatomical axis that was considered the closest to the optimal 3D position.
Fig II.10  Standardized diagram summarizing optimal insertion point positions with respect to ventral anatomical landmarks.

Table II.5  Definitions of optimal insertion points.
Fig II.11 summarizes the referential axes used for all implant sites as well as the conventions used to orient the angles in space. The calculated data independent from implant size (ProjA, widths and lengths) are summarized in Table II.6 and Fig II.12.

**Fig II.11** Diagrams representing ProjA used to define the 3D position of each optimal implant with respect to anatomical axes and the conventions used to orient these angles in space. OSIC are identified using the same color coding as in Table II.6.
Table II.6  Summary of calculated ProjA, widths and lengths for each OSIC in the 3 studied groups.
*: Significantly different from AAI group; ^: Significantly different from N-Toy group.
#: Median values used for non-normally distributed datasets.

<table>
<thead>
<tr>
<th>Implant site</th>
<th>Group</th>
<th>Sagittal ProjA Mean±SD (°)</th>
<th>Transverse ProjA Mean±SD (°)</th>
<th>Dorsal ProjA Mean±SD (°)</th>
<th>Cranio-caudal Width Mean±SD (mm)</th>
<th>Ventrodorsal Width Mean±SD (mm)</th>
<th>Lateromedial Width Mean±SD (mm)</th>
<th>Length Mean±SD (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1 (C1 Pedicular)</td>
<td>AAI</td>
<td>21.06 ±7.34</td>
<td>21.28 ±5.00</td>
<td>± 6.05 ±2.00</td>
<td>± 2.80 ±1.95</td>
<td>7.26 (6.53) ±1.76</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N-Toy</td>
<td>28.46* ±5.63</td>
<td>20.76 ±5.49</td>
<td>± 6.97 ±1.60</td>
<td>± 3.26 ±1.68</td>
<td>8.36 (8.15*) ±1.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N-Beagle</td>
<td>23.9^ ±15.03</td>
<td>16.89** ±15.77</td>
<td>± 10.08** ±11.36</td>
<td>± 3.8** ±10.70</td>
<td>11.03 (10.99**) ±1.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-3 (C1-C2 Transarticular)</td>
<td>AAI</td>
<td>26.32 ±16.37</td>
<td>± 34.44 ±6.35</td>
<td>± 5.26 ±1.85</td>
<td>± 3.70 ±10.53</td>
<td>20.69** ±1.57</td>
<td>3.29 ±1.40</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N-Toy</td>
<td>45.05 ±15.30</td>
<td>± 52.66 ±6.54</td>
<td>± 4.81* ±0.84</td>
<td>± 2.05 ±0.70</td>
<td>8.36 (8.15*) ±1.57</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N-Beagle</td>
<td>42.46 ±13.39</td>
<td>± 47.35* ±4.99</td>
<td>± 7.48* ±1.02</td>
<td>± 3.70 ±10.53</td>
<td>20.69** ±1.57</td>
<td>3.29 ±1.40</td>
<td></td>
</tr>
<tr>
<td>6-7 (C2 Pedicular)</td>
<td>AAI</td>
<td>28.46* ±5.63</td>
<td>20.76 ±5.49</td>
<td>± 6.97 ±1.60</td>
<td>± 3.26 ±1.68</td>
<td>8.36 (8.15*) ±1.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N-Toy</td>
<td>45.05 ±15.30</td>
<td>± 52.66 ±6.54</td>
<td>± 4.81* ±0.84</td>
<td>± 2.05 ±0.70</td>
<td>8.36 (8.15*) ±1.57</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N-Beagle</td>
<td>42.46 ±13.39</td>
<td>± 47.35* ±4.99</td>
<td>± 7.48* ±1.02</td>
<td>± 3.70 ±10.53</td>
<td>20.69** ±1.57</td>
<td>3.29 ±1.40</td>
<td></td>
</tr>
</tbody>
</table>

Table II.6: Summary of calculated ProjA, widths and lengths for each OSIC in the 3 studied groups.
*: Significantly different from AAI group; ^: Significantly different from N-Toy group.
#: Median values used for non-normally distributed datasets.
Fig II.12  Box plots (4 quartiles) of each OSIC characteristics independent from implant size (also see next page).

Purple: AAI group, Orange: N-Toy group, Turquoise: N-Beagle group.

*: Significantly different from AAI group; ^: Significantly different from N-Toy group; (red characters: non-normally distributed datasets).
SafA are defined with respect to each optimal implant placement and therefore they are independent from anatomical axes. CritSafA and CritTecA were identified and calculated for each OSIC and each implant size in all dogs. These critical angles of safety are depicted in Fig II.13. The calculated critical safety angles for the 3 studied implant sizes and 3 groups, as well as the results of intergroup statistical comparisons are presented in Table II.7 and Fig II.14.

**Fig II.13**  *Diagrams representing SafA used to characterize the safety profile of each OSIC.*

Continuous lines: optimal implant positions, Dashed lines: SafA (4/OSIC), Arced surfaces: CritSafA, Arced lines: CritTecA, OSIC are identified using the same color coding as in Table II.7. Note that SafA are not actually projected on anatomical planes, therefore the diagrams are an approximate representation.
### Implant site

<table>
<thead>
<tr>
<th>Group</th>
<th>Implant size (mm)</th>
<th>CritSafA Mean±SD (°)</th>
<th>CritTecA Mean±SD (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAI</td>
<td>1.50 6.48 (3.95)</td>
<td>13.98 ±7.26</td>
<td>14.35 ±6.83</td>
</tr>
<tr>
<td></td>
<td>2.00 3.76</td>
<td>16.20 ±7.75</td>
<td>17.47 ±7.09</td>
</tr>
<tr>
<td></td>
<td>2.40 1.57</td>
<td>16.39 ±7.94</td>
<td>18.24 ±8.24</td>
</tr>
<tr>
<td>N-Toy</td>
<td>1.50 7.16 (6.65)</td>
<td>16.43 ±7.36</td>
<td>17.65 ±5.36</td>
</tr>
<tr>
<td></td>
<td>2.00 4.95</td>
<td>11.60 ±5.72</td>
<td>13.35 ±4.06</td>
</tr>
<tr>
<td></td>
<td>2.40 3.17</td>
<td>13.73 ±6.71</td>
<td>16.06 ±6.06</td>
</tr>
<tr>
<td>N-Beagle</td>
<td>2.00 6.16</td>
<td>12.80 ±8.53</td>
<td>12.52 ±9.23</td>
</tr>
<tr>
<td></td>
<td>2.40 4.71 (5.50)</td>
<td>10.42 ±16.64</td>
<td>11.00 ±10.63</td>
</tr>
</tbody>
</table>

### Implant site

<table>
<thead>
<tr>
<th>Group</th>
<th>Implant size (mm)</th>
<th>CritSafA Mean±SD (°)</th>
<th>CritTecA Mean±SD (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAI</td>
<td>1.50 16.39 (14.35)</td>
<td>25.94 (15.09)</td>
<td>29.94</td>
</tr>
<tr>
<td></td>
<td>2.00 13.89</td>
<td>21.42 ±7.09</td>
<td>18.28 ±6.84</td>
</tr>
<tr>
<td></td>
<td>2.40 11.24</td>
<td>10.95 ±6.14</td>
<td>11.64 ±2.45</td>
</tr>
<tr>
<td>N-Toy</td>
<td>1.50 14.28 (14.71)</td>
<td>12.70 (12.47)</td>
<td>14.87</td>
</tr>
<tr>
<td></td>
<td>2.00 11.92</td>
<td>8.37 ±4.92</td>
<td>14.92</td>
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<td>2.40 10.07</td>
<td>13.59 ±3.88</td>
<td>15.00</td>
</tr>
<tr>
<td>N-Beagle</td>
<td>2.00 15.84 (14.63)</td>
<td>11.00 (10.33)</td>
<td>15.35</td>
</tr>
<tr>
<td></td>
<td>2.40 14.31 (14.97)</td>
<td>13.32 ±7.52</td>
<td>15.40</td>
</tr>
</tbody>
</table>

### Table II.7

Summary of calculated CritSafA and CritTecA for each OSIC and each implant size in the 3 studied groups (see next page).

Purple: AAI group, Orange: N-Toy group, Turquoise: N-Beagle group.

*: Significantly different from AAI group/1.5mm implant; ^: Significantly different from N-Toy group/1.5mm implant; (red characters: non-normally distributed datasets).

Fig II.14 Box plots (4 quartiles) of each OSIC CritSafA and CritTecA calculated with 3 different implant sizes (next 2 pages).

Purple: AAI group, Orange: N-Toy group, Turquoise: N-Beagle group.

Light color: 1.5mm implant, Intermediate color: 2mm implant, Dark color: 2.4mm implant

*: Significantly different from AAI group/1.5mm implant; ^: Significantly different from N-Toy group/1.5mm implant; (red characters: non-normally distributed datasets).
IV. Discussion

This study provided a detailed description of a new method of AA OSIC analysis using OsiriX™. To the authors’ knowledge, this is the first extensive anatomical description of AA OSIC in dogs. Multiple surgical techniques have been reported in the literature; however technical descriptions of optimal implant positions have been vague. For most bone corridors, optimal implants have been depicted using diagrams but only rarely precise landmarks or numerical values were provided (Platt, Chambers, et al. 2004, Aikawa, Shibata, et al. 2013, Dewey 2013). The main AA OSIC that has been prospectively studied in dogs is the corridor used for transarticular fixation. Sorjonen and Shires provided a radiographic study determining optimal projected angles when they first developed the transarticular technique in 1981 (Sorjonen & Shires 1981). The main anatomical landmark used to define the corridor was the medial border of the alar notch; however an insertion point was not precisely defined. The alar notch has been subsequently reported as a common landmark for AA transarticular fixation (McCarthy, Lewis, et al. 1995, Aikawa, Shibata, et al. 2013). However, in a recent publication studying AA transarticular bone corridor, the authors elected to use a different definition of the optimal implant placement (Vizcaino Reves, Stahl, et al. 2013). The point of insertion was defined as “the deepest point of the pit medial to the cranial end of the crest running from the transverse process to the articular process”, while the exit point of the corridor was defined as “the cranial opening of the transverse foramen of the atlas”. It is important to recognize that optimal implant positions provided in the literature have been subjectively defined, and therefore previously reported values have to be used cautiously, taking into consideration the provided definition of the bone corridor.

The method of OSIC determination described in the present study allowed overcoming the problem of subjective optimal implant definitions. 3D simplification of
bone corridors into geometrical shapes not only permitted the description of corridors of complex/oblique distribution, but also to objectively localize the optimal implant position in space. Combining this novel approach with precise mapping of the ventral surface of the AA joint using landmarks available to the surgeon, we were able to generate objective definitions of optimal insertion points. Only 2 of the OSIC insertion points were not geometrically determined due to their predefined sagittal position. For the transarticular OSIC, the geometrical determination of the corridor centered axis was based on the C1 lateral masses while the insertion point was located on the C2 cranial articular surface. In order to obtain an accurate description of the transarticular insertion points, a correction of AA misalignment had to be performed using C1 and C2 screen copies of the 3D-VR mode. This problem had previously been described in a recent CT study and precluded those authors from determining optimal implant placement in dogs suffering from AAI (Vizcaíno Reves, Stahl, et al. 2013). In that particular study, angular measurements had been manually performed in 3D-MPR mode which did not allow correction of misalignment. To our knowledge, the results of the present study represent the first report of AA optimal implant placement in dogs suffering from AAI. Our method of misalignment correction was still somewhat imprecise, but the fact that we obtained identical optimal insertion point definitions for both transarticular and C2 cranial articular surface through 2 independent methods suggests that the 30° line truly represents a centered point of the C2 cranial articular surface.

For the determination of numerical values describing 3D optimal implant placements and bone corridor characteristics, we developed a semi-automated procedure relying on the 3D coordinates of pre-identified ROI points. The mathematical calculations and data transfer was successfully validated via concordance analysis between calculated and manually measured values. Our data also implied that SafA and ProjA could be accurately obtained using OsiriX™ measurement tools, although this was more time
consuming compared to the semi-automated method.

Other potential sources of error for the determination of OSIC characteristics were identified. Inaccuracy when positioning the ROI points simulating either the optimal implant, the safety margins or representing the anatomical axes were considered. A theoretical error in any ROI positioning was expected up to half the CT study slice thickness (0.3125mm) which would be expected to have a low impact on most of the OSIC calculated values. This error could have been minimized by reformatting all CT studies to 0.1mm slice thickness in OsiriX™. Using concordance analysis, we were also able to estimate the error induced by the use of the described landmarks for anatomical axes simulation. This analysis revealed an overall low 95% tolerance interval [-1.62°, 1.61°], which was slightly wider when comparing inter-observer values of ProjA calculated in C1 coordinate system [-3.58°, 3.72°]. Overall the error generated by the anatomical axes simulation was considered small and it should be emphasized that such error cannot be avoided when using ProjA values as 3D coordinates. Based on our results, this error can be significantly reduced if 2 observers position the ROI points successively and the mean of the 2 obtained ProjA values is used instead of an individual value. The use of OsiriX™ with implementation of mathematical equations on 3D ROI coordinates was therefore considered an efficient and very reproducible tool. Some occasional issues were encountered when placing ROI points on 3D-VR mode in some cases but they did not preclude successful OSIC simulation in any case. The major advantage of the described method was its ability to generate 3D data defined with respect to anatomical coordinates. Such data could be used for applications beyond OSIC descriptions. For instance, 3D data can be used to study the biomechanics of complex motions between vertebral motion units or to characterize thresholds of pathological range of motion such as observed in AAI.
ProjA values can be directly used in practice for clinical cases to define the 3D position of the optimal implants. In surgery, an optimal insertion point represents the point of initial drilling while the 2 ProjA define the direction of drilling. Several limitations should be considered before applying the provided data to a patient. First, the ranges of ProjA values for most OSICs ranged between 20-30°. This would imply that using the mean or median value for all cases would potentially cause positioning error of up to 10-15°, assuming the surgeon is able to place the implant in its intended position. Such misplacement could be disastrous in some instances, especially in case of narrow safety margins like transarticular implants. Therefore, the use of these reported means or median values should be reserved for OSIC with wider safety margins. Second, it is important to remember that ProjA are defined with respect to anatomical axes. Consequently, a good understanding of these definitions and how projections are made is essential for successful implant positioning. Identification of the sagittal plane is the most intuitive step to define a ProjA as it is also the plane of symmetry of the vertebra. An anatomical axis is then needed within that plane to define a proper coordinate system. In a previous report, the craniocaudal axis for C2 was defined as the ventral border of the vertebral foramen, which is what was also used in our study (Vizcaino Reves, Stahl, et al. 2013). To our knowledge, a similar axis had not yet been described for C1. Therefore, we subjectively defined the cranial border of the dorsal and ventral arches as representing the ventrodorsal axis. The implicit assumption was that these C1 and C2 anatomical axes would have good alignment when the AA joint is placed in neutral position. Further investigation on the neutral position of the AA joint would be necessary to assess the accuracy of that assumption. Anatomical axes misalignment could result in clinically significant consequences when placing C1 implants. Indeed, only a very small portion of C1 can be visualized intraoperatively which means that estimation of C1 implant 3D position in surgery is mostly based on landmarks present on
the ventral surface of C2. Fortunately this type of misalignment would have limited impact on the risk of vertebral canal violation (except for a C1 ventral arch implant) as it would only affect the implant direction along the sagittal plane, not lateromedially.

The detailed values of ProjA and associated orientation conventions presented here are difficult to compare to the data provided by the literature due to different OSIC definitions and different referential axes. For instance the ProjA values for the transarticular implant in N-Toy were 30.10±4.93° for sagittal ProjA and 32.01±5.54° for dorsal ProjA, while a previous study obtained 20±1° and 40±1° respectively (Vizcaino Reves, Stahl, et al. 2013). These differences are not necessarily surprising given that the definition of optimal implant placement was different (not aiming at the alar notch) and the ProjA values were measured using C2 projections instead of C1. However, the very low standard deviation reported in that study was more unexpected given the wide variations observed in our population. It is possible those authors identified a corridor with minimal individual variation, or maybe the method of angle measurement carried a systematic bias. In 1981, Sorjonen also reported transarticular ProjA values (21.5° and 29°) based on measurements obtained in 18 mixed breed dogs using radiographs, which were slightly closer to the results obtained in our Beagle group (29.43±3.66° and 30.72±2.57°). Similar to the previous CT study, they used a C2 projection which implies that any misalignment between C1 and C2 at the time of imaging may have modified the ProjA values. Another study reported a value of 50° (range: 45-60°) for the transverse projected angles of C2 pedicular screws as part of a CT spinal study involving dogs of various sizes (Watine, Cabassu, et al. 2006). The values reported in the current study are in the 30° range which can again be explained by significant differences in optimal implant definitions. In that study the authors were aiming at preventing violation of the vertebral arteries in the transverse foramen, while this was considered impossible in Toy breeds and Beagles due to their small size. Moreover, this previous study depicted
pedicular implants as also penetrating the vertebral body while the OSIC we used only aimed at bone purchase within the pedicle and transverse process. These differences highlight the importance of correlating reported ProjA values with precise definitions of OSIC.

CT measurements of the transarticular safe corridor width have been previously described and ranged from 3 to 4.5mm for dogs less than 5kg (Vizcaino Reves, Stahl, et al. 2013). Based on that result, the authors recommended using screws of 1.5-2mm diameter in toy breed dogs. The calculated values of lateromedial width in our study revealed to be within a similar range for most of the studied OSICs except for the Beagle group, which generally had significantly wider corridors. In order to objectively justify a recommendation on implant diameters, we proposed a method of SafA calculation that considered the size of implant used obtaining data represented as CritSafA and CritTecA. CritSafA should be prioritized given that it represents the safety margin preventing vertebral canal violation. Knowing that significant individual variations of ProjA were previously noted and considering the potential for surgical inaccuracies, it would seem reasonable to have at least 15-20° safety margins before vertebral canal violation occurs. Based on our data, this degree of safety would be difficult to obtain for most bone corridors if the implant was larger than 1.5mm, although the C2 cranial articular surface and pedicular OSICs would represent the main exceptions. Therefore, from a safety standpoint, using implants larger than 1.5mm in Toy breed dogs should be avoided unless an individual CT study is obtained justifying larger implants. Biomechanical studies would however be necessary to further support that statement, as smaller implants may negatively impact the strength of the stabilization construct.

Overall, the SafA data provided in this study can be used to compare the relative safety of different OSICs. For instance, C1 implants and transarticular implants have particularly narrow safety margins that a neurosurgeon should be aware of. For the
implants located in the sagittal plane (monocortical), SafA were calculated based on a theoretical implant of 75% of the total length of the corridor. Consequently, SafA could be increased if necessary by using a shorter length. Further investigation would be necessary to determine the ideal length of monocortical implants that would provide sufficient bone purchase with optimized SafA.

Statistical comparisons were conducted between groups to determine whether N-Toy and N-Beagles could be considered appropriate models of AAI cases. Even though some values of ProjA were statistically different between groups, the differences were generally small and considered unlikely to be clinically relevant. Accordingly, our data suggest that both N-Toy and N-Beagles would be considered appropriate model in terms of geometrical distribution of the OSICs compared to AAI dogs. In regards to the widths and lengths measurements, N-Beagles often had significantly larger values, as expected from the difference in body size. Surprisingly, the lateromedial width of the transarticular OSIC was not significantly wider in N-Beagles compared to the other 2 groups. It seemed that the lateral masses of Beagles were proportionally narrower compared to the other 2 groups. This was also supported by intergroup comparisons of CritSafA for both C1 pedicular and transarticular OSICs, which revealed very similar safety profiles using implants of the same diameter in all 3 groups. This morphological variation could be significant if N-Beagles were used to study the accuracy of implant placement.

The recruitment of AAI cases proved to be difficult within our hospital population. Only 5 cases were recruited within the study period while we initially aimed at 10. Furthermore, 2 of the AAI CT scans were acquired with an increased slice thickness (1.25mm) due to technical error which may have slightly reduced the precision of ROI point positioning in OsiriX™. Therefore the data presented for the AAI group should be interpreted with caution. The risk of poor recruitment of AAI cases had been anticipated
at the time of study design which is why N-Toy dogs were also recruited. The typical criteria that has been used in previous studies focusing on Toy breed dogs is usually a body weight <5g (Vizcaino Reves, Stahl, et al. 2013). This criteria revealed to be practical in terms of sample recruitment, although the body weight of the sampled N-Toy was slightly increased compared to the AAI group (3.32±1.15Kg vs. 2.32±1.09Kg; p=0.0121). It should therefore be considered that our results may be more applicable to AAI cases in the higher range of body weight (2-5kg). Interestingly, the N-Toy dog with the lowest body weight in our study (0.9kg) revealed to have a distribution of insertion points in the caudal vertebral body of C2 that seemed significantly different from the rest of the sampled population. Our data did not allow determining if smaller Toy breed dogs have a different vertebral morphology but this case brings up that possibility. It should be noted that this dog was also the youngest of the sampled population (5.5 months) and therefore a different growth stage or individual anatomical variation could not be excluded. Nevertheless, clinicians should be aware of such individual variations and apply our results cautiously in very small dogs. Other individual morphological variations of C1 have previously been associated with AAI in dogs. One study reported that incomplete atlas ossification was significantly correlated to AAI (Parry, Upjohn, et al. 2010). The presence of such individual morphological variations is a major justification in human medicine to implement individualized surgical planning as well as intraoperative neuronavigation, particularly in pediatric patients or those suffering rheumatoid arthritis, where anatomical variations are highly expected (Chung, Lee, et al. 2006, Takahashi, Shono, et al. 2007, Chern, Chamoun, et al. 2009).

Neuronavigation can be defined as a group of techniques designed to target neuroanatomical structures intraoperatively using 3D data obtained via advanced imaging. These methods include frame-based systems where the 3D space is defined with respect to a rigid frame, and frameless systems which requires more advanced
intraoperative 3D calculations, but allows for greater freedom of movement for the surgeon (Wininger 2014). In veterinary medicine, neuronavigation represents a promising technical advancement in surgical accuracy, even though it remains cost-prohibitive for most clinical practices. The main alternative to neuronavigation is precise pre-surgical planning. The free open-source DICOM software OsiriX™ was initially developed by a human radiologist to improve multidimensional navigation and display of large datasets generated by advanced imaging modalities such as PET-CT or Cardiac CT (Rosset, Spadola, et al. 2004). OsiriX™ rapidly became a successful 3D navigation tool due to its extremely fast and optimized 3D graphics. More recently, several publications in human medicine have reported its use for fast pre-surgical planning, notably in emergency situations where advanced neuronavigation planning is considered excessively time consuming (Fortin & Battie 2012, Jaimovich, Guevara, et al. 2014). In our study, OsiriX™ proved relatively intuitive, and overall easy to use. The software could easily be complemented with pre-surgical planning tools through the development of plugins incorporating mathematical equations similar to those we used in our study. This would allow generating optimal implant placement definitions considering individual morphological variations of the patient. The precise calculation of such coordinates may improve surgical accuracy, however, this would require the development of intraoperative guiding systems able to use these values to position the implants in vivo.
V. Conclusion

In conclusion, this study described a novel semi-automated method of OSIC analysis using OsiriX™ DICOM viewer software. Geometrical simplification of bone corridors generated objective definitions of optimal insertion points that were similar for the 3 studied groups. The method of calculation of ProjA and SafA was successfully validated by comparing the results to manually measured values. The mathematical model could be used successfully to determine AA OSIC characteristics in our sampled population, which was its primary purpose. However, it could also be further developed as an interactive preoperative/intraoperative planning tool for individualized surgical planning of spinal implants. Individual anatomical variations are becoming more commonly recognized in the canine population, and therefore the need for individualized pre-surgical planning will likely be increasing. Neuronavigation has been rapidly expanding in human medicine, but such advanced technology remains currently inaccessible to most veterinary practices. While continuous imaging guidance is definitely an appealing solution to many challenges associated with vertebral implant placement, it is the authors’ belief that satisfactory accuracy could also be achieved using simple ProjA values and precise definitions of optimal implant placement.
VI. References


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CHAPTER III - BIOMECHANICAL AND RADIOLOGICAL COMPARISON BETWEEN TRANSARTICULAR SCREW FIXATION AND 2 MULTI-IMPLANT CONSTRUCTS FOR VENTRAL ATLANTOAXIAL STABILIZATION

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I. Introduction

Canine atlantoaxial (AA) instability is a common cause of cervical myelopathy in young toy breed dogs (Cerda-Gonzalez & Dewey 2010, Plessas & Volk 2014). Severity of clinical signs may vary from chronic pain to acute respiratory arrest and sudden death (Cerda-Gonzalez & Dewey 2010, Parent 2010). The presence of AA instability is typically demonstrated using radiographs or advanced imaging modalities such as computed tomography (CT) or magnetic resonance imaging, however there is currently no established grading system predicting future progression of the disease (Westworth & Sturges 2010, Middleton, Hillmann, et al. 2012, Vizcaino Reves, Stahl, et al. 2013, Plessas & Volk 2014, Stalin, Gutierrez-Quintana, et al. 2015). Other sites of vertebral column instability are evaluated using the “3-column” system to help clinicians decide whether surgical stabilization is indicated or not (Jeffery 2010). Similar guidelines are lacking for canine AA instability and therapeutic decisions are often based on subjective assessment of the patient. Temporary immobilization of the craniocervical junction with external bracing can be attempted but relapse or acute worsening is commonly reported with this approach (Havig, Cornell, et al. 2005). In this context, surgical stabilization is often considered the best therapeutic approach, even though technically challenging. This is reflected by the fact that in the past 50 years, most of the literature on the subject has been focused on developing and improving AA stabilization techniques (LeCouteur, McKeown, et al. 1980, Sorjonen & Shires 1981, Kishigami 1984, Schulz, Waldron, et al. 1997, Dickomeit, Alves, et al. 2011, Aikawa, Shibata, et al. 2013, Sanchez-Masian, Lujan-Feliu-Pascual, et al. 2014, Stalin, Gutierrez-Quintana, et al. 2015).

Failure of the atlanto-odontoid system is well recognized as the main cause of AA instability and was documented in 2 recent biomechanical studies (Reber, Burki, et al. 2013, Zang, Liu, et al. 2015). Therefore, the objective of AA stabilization is to restore the
normal function of the atlanto-odontoid system, which is to provide flexible support of the head, without significantly impairing the vital structures traveling through or in close proximity to the craniocervical junction. From a biomechanical perspective, this equates to bringing the pathologically increased range of motion of the AA motion unit within the normal pain free zone (Panjabi 1992b). Theoretically, this could be achieved through dynamic systems such as atlanto-odontoid joint replacement, but, to the authors’ knowledge, such systems have only been used experimentally (Zang, Liu, et al. 2015).

In current clinical practice, stabilization is achieved through rigid fixation of the AA motion unit to obtain intervertebral bony fusion (arthrodesis). Because bony fusion is the main purpose, biomechanical constructs with high stiffness and minimal range of motion are usually preferred. In other words, the biomechanical goal of current AA stabilization techniques is to achieve rigid stabilization to facilitate arthrodesis of the joint.


There is currently very little data comparing biomechanical properties of AA stabilization constructs. Dorsal suture stabilization has been established to have lower stiffness than multi-implant constructs, but biomechanical properties of different ventral techniques have yet to be compared in terms of strength and stiffness (Kopf, Elder, et al. 2013). In contrast, multiple studies have compared the biomechanical characteristics of AA stabilization techniques in humans (Grob, Crisco, et al. 1992, Hanley & Harvell 1992, Wilke, Fischer, et al. 1992a, Wilke, Fischer, et al. 1992b, Menezes & Traynelis 2008, Elliott, Tanweer, et al. 2012). Surgical techniques are similar to those employed in dogs except that implants are typically placed dorsally. Experimental work has demonstrated that TSF technique and internal fixation with multiple screws combined with rods offer the most rigid fixations and constitute the preferred methods in people (Menezes & Traynelis 2008). Dorsal suture techniques are indicated when a semi-rigid system is beneficial (pediatric medicine) or when anatomical anomalies do not allow pedicle screw placement. However, these semi-rigid techniques require concurrent external orthosis and are associated with a lower rate of arthrodesis (Ahmed, Traynelis, et al. 2008). Experimental biomechanical data is important to consider when comparing stabilization methods and is currently lacking in dogs.

The other major aspect of AA stabilization technical outcome is the potential for iatrogenic disruption of vital structures. In human medicine, extensive precautions are taken to avoid both vertebral canal violation and vertebral artery injury when performing
cervical and AA instrumentation (Kamimura, Ebara, et al. 2000, Kazan, Yildirim, et al. 2000, Ludwig, Kramer, et al. 2000, Igarashi, Kikuchi, et al. 2003, Chung, Lee, et al. 2006, Fu, Lin, et al. 2013). Despite using advanced techniques of preoperative planning and intraoperative guiding systems, implant misplacement can still be occasionally identified on postoperative CT (4.4-7%), although only very few cases are considered clinically significant (0.25-0.34%) (Elliott, Tanweer, et al. 2012). In veterinary medicine, the rate of vertebral canal violation associated with AA stabilization is unknown. Postoperative CT with thorough assessment of implant placement is seldom reported in the literature and the sensitivity of postoperative radiographs to detect vertebral canal violation has been demonstrated to be poor (Hettlich, Fosgate, et al. 2010). Canine cadaveric studies have shown that cervical ventral implant placement is challenging with vertebral canal violation rates of 9.7% for monocortical implants and as high as 62.5-100% for bicortical implants (Hicks, Pitts, et al. 2009, Hettlich, Allen, et al. 2013). Even though experimental data is lacking regarding AA stabilization, it can be anticipated that even greater technical difficulties exist in this region, considering that most dogs suffering from AA instability are of extremely small size (Plessas & Volk 2014). The quality of anatomical reduction after stabilization of an AA subluxation is another parameter which has never been reported and should be considered when assessing the technical outcome.

The objective of this study was to prospectively describe and compare the biomechanical properties, quality of anatomical reduction and safety profile of 3 commonly used methods of ventral AA stabilization in canine cadavers. It was hypothesized that all 3 techniques would provide equivalent strength and stiffness in ventral bending and similar preservation of vital structures.
II. Materials and methods

Sample size and random assignment of Beagle cadavers

Based on standardized spinal implant biomechanical testing recommendations and previous veterinary studies comparing different methods of spinal stabilization, a sample size of 7 cadavers for each tested stabilization technique was elected (Garcia, Milthorpe, et al. 1994, Wilke, Wenger, et al. 1998, Hicks, Pitts, et al. 2009, Hettlich, Allen, et al. 2013). The cadavers of 21 mature Beagle dogs euthanized for reasons unrelated to this study and donated to the Ontario Veterinary College for teaching and research purposes were recruited. The dogs were maintained frozen at -20°C until they were used for surgical AA stabilization. The cadavers were randomly ordered by generating a random permutation of 21 numbers. Each dog was then assigned a group number corresponding to the 3 compared AA stabilization methods. This was achieved using restricted random assignment to insure an equal sample size of 7 dogs per group.

Ventral atlantoaxial stabilization surgical techniques

The available literature was reviewed to determine the most commonly reported ventral AA stabilization techniques. Based on this information, we selected 3 methods of AA stabilization including TSF (using 2 transarticular cortical bone screws) and 2 multi-implant constructs using respectively 5 cortical bone screws (MI5) or 6 cortical screws (MI6) placed in C1 or C2 and embedded in PMMA cement (Fig III.1).

The cadavers were thawed at room temperature prior to the surgery and carefully positioned in dorsal recumbency using a vacuum positioning system (Vacu-Positioner Pad® Shor-Line, Kansas City, KS, USA). Then the ventral surface of C1 and C2 was surgically approached using the paramedian method previously described by Shores in
2007 (Fig III.2.A). The AA joint was subsequently destabilized by burring through the base of the dens of C2 resulting in odontoid separation (Fig III.2.B). Once destabilized, the vacuum positioning system was disconnected and the dog was moved away from the surgical table to be once again positioned afterwards. This repositioning would allow simulating typical AA subluxation observed intraoperatively. Gelpi retractors were then positioned at the level of the hypaxial muscles to laterally retract the paired longus colli and longus capitis muscles as well as more superficial anatomical structures.

**Fig III.1** Computed tomography 3D reconstruction of the 3 tested stabilization constructs in ventral and right lateral views.
TSF: Transarticular fixation consisting of 2 bone screws placed through the AA joint.
MI5: Multi-implant construct using 5 bone screws including 2 in C1 lateral masses, 2 in C2 cranial articular surface and 1 in C2 caudal vertebral body.
MI6: Multi-implant construct using 6 bone screw similar to MI-5 except the single screw in C2 caudal vertebral body is replaced by 2 C2 pedicular screws.
Green areas: PMMA cement embedding bone screws.
Fig III.2 Surgical approach of the AA joint and odontoid separation performed prior to AA stabilization.

(A) Superficially, a paramedian approach was used displacing all vital structures to the left (green arrow); at the level of the hypaxial muscles, a median incision (green dashed line) allowed to separate the paired longus colli (l.c.) and longus capitis. (B) The base of the odontoid process was burred using an electric handheld rotatory tool (Dremel® Robert Bosch Tool Corp., Mount Prospect, IL, USA) until dens separation was achieved.

Once the ventral vertebral surfaces were visualized, the implant insertion points were identified using pre-established definitions of the optimal implant position. These definitions were based on CT analysis of optimal safe implantation corridors presented in chapter II (Fig II.10 & Table II.5). Insertion points were physically marked on the bone surface by pre-drilling the cis-cortex (insertion cortex). The results of the same CT study were also used to define the 3D direction of drilling required for screw implantation. In that study, 3D positions were defined using 2 projected angles with respect to anatomical planes (sagittal, transverse or dorsal). The mean of the reported projected angle values were used, therefore the same 3D position was intended in all dogs for a specific implant site. In order to optimize our ability to reproduce these values while performing the surgery in situ, we developed a 3D drill guide prototype enabling the
surgeon to align the drill with the theoretical optimal axis. Alignment of the drill guide was based on Cartesian coordinates of the optimal implant direction vector which were calculated using previously determined optimal projected angles (Table III.1 & Fig III.3). Even though the drill guide was used for all implants, the exact direction of drilling was visually adjusted for the TSF technique, always aiming toward the alar notch (Fig III.3.D). This slight intraoperative correction was applied because the data from the previous CT study suggested that lateromedial angular safety margins for TSF implants were too small to use a single optimal 3D position for the general Beagle population.

Table III.1 Coordinates used to align the 3D drill guide with optimal implant positions. This table was generated to facilitate the use of the drill guide intraoperatively. Each implant site and side corresponds to a 3D coordinate value (X, Y, Z). The cell color for each coordinate refers to the vertical bar it applies to (silver or gold colored handle). Coordinates were set up so that the drill was always inserted through the vertical bar with the gold colored handle. To generate these values, a distance had to be subjectively fixed. We elected to set a distance between the 2 vertical bars along the drilling axis that would allow appropriate drilling lengths for the different implants (see also Fig III.4.E). Trigonometric equations were used to calculate X, Y and Z values based on the 2 projected angles and the distance between the 2 vertical bars. * Projected angle values extrapolated from previous CT study (Beagle group).
Fig III.3 Diagram and pictures demonstrating the use of the 3D drill guide prototype to align the drilling axis with the theoretical optimal implant position.

(A) The drill guide uses a Cartesian 3D coordinate system which can be aligned with anatomical planes (sagittal in orange, transverse in purple). Previously established projected angle values ($\theta_y$) can be used to determine x,y,z values (see Table III.1).

(B) Once the (x,y,z) coordinates are set up on the drill guide, the (x) and (z) axes are visually aligned with the craniocaudal and right to left anatomical axes respectively. The guide is maintained in this position while drilling through the vertebrae.

(C) The drill guide also protected the surrounding soft tissues from iatrogenic injury.

(D) For the TSF technique, the alar notch was identified using a small instrument (arrow) and the drilling direction was adjusted to aim toward the instrument (dashed line).

Note: the graduated rods used for precise positioning of the (x, y, z) coordinates were machined from commercially available optic cage systems: Cage Assembly Rod Ø6 mm (Thorlabs Inc., Newton, NJ, USA).
The positions of the stabilizing implants within the surgical field of view are presented in Fig III.4.A-C. All implants used for stabilization were self-tapping cortical screws of 2.4mm diameter, 1.0mm thread pitch and 1.7mm core diameter (DePuy Synthes, West Chester, PA, USA). As recommended by the manufacturer, a 1.8mm drill bit was used for all implantation sites. The length of the implants was planned based on previous CT data. For TSF implants, 26-28mm screws were used so that each screw was placed through both cortices with at least 2mm protruding from the trans-cortex (exit point). For bicortical implants embedded in PMMA, the screw length was estimated to allow at least 2mm beyond the trans-cortex with their heads protruding approximately 8mm from the cis-cortex resulting in 24-26mm and 12-16mm screw lengths for C1 and C2 implants respectively. For monocortical sagittal implants in the MI5 technique, a 6mm drilling length (75% of the bone corridor) and total screw length of 14mm was used. The previously described drill guide was used to control the drilling depth precisely (Fig III.4.E).

With the TSF technique, reduction of the AA joint was achieved using a towel clamp placed between C1 and C2 (Fig III.4.D) which could be maintained while drilling and positioning screws. This method was not applicable for the MI5 and MI6 groups due to the presence of PMMA cement (Palacos® R, Zimmer, Inc., Warsaw, IN, USA). Instead, lateral tension applied to the hypaxial muscles with Gelpi retractors resulted in anatomical reduction of the AA joint assessed visually. This tension could be maintained while PMMA cement was positioned to embed all implanted screws (Fig III.4.F). The quantity of PMMA cement used in all dogs was normalized to ¼ dose (10g of powder mixed with 5mL of liquid).

Once the surgery was completed, the stabilized AA joint, composed of the C1 and C2 vertebral segment, was dissected, extracted, wrapped with gauzes and soaked with
0.5% NaCl solution. All samples were then protected from dehydration with 2 successive hermetic plastic bags and stored at -20°C until subsequent testing.

Fig III.4 Pictures demonstrating placement of stabilizing implants in TSF, MI5 and MI6 groups.

(A) Position of the 2 transarticular screws used in TSF technique. (B) Position of the 5 screws used in MI5 technique. Note that all screws protrude approximately 8mm to allow cement embedding. The screw located in C2 caudal vertebral body is the only monocortical implant used (arrow). (C) Position of the 6 screws used in MI6 technique. (D) A towel clamp was used to maintain reduction of the AA joint while drilling and placing the transarticular screws. (E) A drill guide only allowing 6mm of drilling length was used to place the monocortical implants. (F) Tension applied via the Gelpi retractors was used to maintain reduction while applying PMMA cement to embed all screws.

Post-operative computed tomography of stabilized AA joints

CT images of the extracted frozen AA joints were obtained using a 16 slice GE
Brightspeed CT scanner (GE Healthcare, Milwaukee, WI, USA). The raw data was acquired with a standardized protocol in axial mode at 25cm collimation, 1.0s rotation time, 0.625 slice thickness, 1.25mm interval, 120kV and 200mAs using both standard and bone algorithms. The images were then reviewed by 2 of the authors (AZ & GL) including a Board Certified veterinary radiologist using the free open-source software version of OsiriX™ DICOM viewer (OsiriX™ version 5.9, Pixmeo, Geneva, Switzerland). Assessment of the position of the implants, quality of cement embedding, AA apposition and AA alignment was performed using original CT slices, 3D multi-planar reconstruction (MPR) mode and 3D volume rendering (VR) mode. Screw position was subjectively characterized as appropriate, hazardous or dangerous (Fig III.7.A-F). Implants were classified as appropriate when placed successfully within the intended optimal safe implantation corridor. Hazardous position described implants penetrating the atlanto-occipital joint, causing minor violation of the vertebral canal, or achieving questionable bone purchase. Screw position was defined as dangerous if suspected clinically significant vertebral canal violation was observed. Cement embedding was assessed for each screw as either appropriate or questionable if there was significant space without cement surrounding the protruding screws. Alignment was subjectively graded using 3D-VR mode as either appropriate or significantly misaligned if there was suspicion that C1-C2 misangulation in any direction could result in any vital structure impairment. Apposition was assessed using both 3D-MPR and 3D-VR modes and classified as optimal, suboptimal or poor (Fig III.7.G-H). Apposition was graded optimal if physiological vertebral canal diameter was preserved. Suboptimal apposition corresponded to a surgical reduction that caused minimal decrease of the vertebral canal diameter at the level of the AA junction, while poor apposition caused significant decrease of the vertebral canal diameter.

Considering that these definitions of technical outcome were subjective, we elected
to reach a consensus between 2 authors for all assessed parameters. The technical outcome was then compared between different surgical techniques and different implant sites. The quality of screw positioning for 1 technique was numerically graded by the number of hazardous and dangerous screws identified in each dog. For statistical comparison between implant sites, right and left sides were considered the same implantation site.

**Biomechanical testing of stabilized AA joints**

Prior to biomechanical testing each AA stabilized construct was thawed at room temperature and protecting gauzes were removed. Then, any soft tissue structure attaching C1 to C2 was carefully dissected away from the vertebrae. The purpose of biomechanical testing was to determine the ventral bending moment at failure, estimate the stiffness of each stabilization construct and compare TSF, MI5 and MI6 groups. In order to achieve this goal, a custom jig system was designed to hold C1 and C2 vertebrae while applying ventral bending without interfering with any component of the stabilization constructs (Fig III.5). The stabilized AA joints were then positioned in a universal material testing system (Instron® Model 5965, Illinois Tool Works Inc., Instron Co., Norwood, MA, USA) equipped with 5kN load cell used in extension mode. Tension load was converted into ventral bending moment by connecting a metallic chain to the bar holding C2 approximately 14cm away from the axis of rotation creating a bending arm. The exact distance was determined by measuring in each case the longitudinal length of the C2 vertebral body from the base of the dens to the epiphysis using previously obtained CT images. Incremental tension was obtained by displacing the crosshead at a constant speed of 10cm/min. Data was collected while tension was applied at 30ms intervals using Instron data acquisition software (Bluehill®3).
Mathematical equations were determined to convert the raw data of tension loads into ventral bending moments considering weights applied on the load cell and angular position of the bending arm (Fig III.6). These calculations were operated on the raw data using Microsoft® Excel software (version 2011, Microsoft Corp., Redmond, WA, USA) to generate load displacement curves with the ventral bending moment on the y axis and angular displacement on the x axis.

Failure of stabilization constructs was defined as a 5% drop in ventral bending moment. Bending moment at failure was defined as the maximal bending moment value obtained before failure was observed. Stiffness of the stabilization constructs was calculated via linear regression in the 0-5° region and 10-15° region of bending. A ratio between the 2 calculated stiffness values was also determined to assess loss of stiffness occurring with bending. Samples were tested following the same order as for surgical stabilization. Once construct failure was achieved the stabilized joint and jig were both inspected to identify the source of failure. This inspection was subsequently complemented by anatomical preparation of the constructs to remove any remaining soft tissue. The type of failure was recorded and classified as instrumentation failure, bone failure, jig failure or unknown. Any suspicion of hooking system failure (such as deformation) prompted replacement of the hook for the next experiment.
Fig III.5  

Pictures demonstrating mechanical principles of the jig manufactured to hold AA joints for biomechanical testing.

(A) Dorsal overview of the jig. The part holding C1 is composed of an aluminum bar with a rounded extremity apposed to the C1 cranial articular surfaces and 2 hooks placed on the C1 dorsal arch. The part holding C2 is composed of a similar aluminum bar with a triangular extremity apposed to the C2 caudal epiphysis and a central stainless steel bar used to hook the C2 lamina. (B) Both hooking systems function with the same mechanical principle. Each hook has a threaded extremity where handheld nuts can be tightened (purple arrows). This generates compressive forces on each extremity of the vertebrae (orange arrows) holding C1 and C2 in place. Tempered drill rods were used to manufacture C1 hooks in order to maximize their resistance to bending. (C) Zoomed dorsal view of both hooking systems placed on C1 dorsal arch and C2 lamina. (D) Right lateral view of the jig demonstrating how ventral tension applied on the bar holding C2 generates ventral bending moment (VBM) on the tested construct.
Fig III.6 Diagram demonstrating equations used to calculate ventral bending moment (VBM) values based on measured tension ($T_c$) and displacement of the crosshead ($y$).

A: Axis of rotation; B: Center of gravity of the bar holding C2; C: Point of insertion of the chain; $\theta$: Angulation of the bending arm with respect to the horizontal position; X: Orthogonal distance from the axis of rotation; Y: Vertical displacement of the crosshead with respect to the 0° position; $L_{C2}$: Length of C2 vertebral body (measured on CT images); $L_B$: Distance between B and C2 (0.0925m); $L_C$: Distance between C and C2 (0.12m); $W_B$: Weight of the bar; $m_B$: Mass of the bar (0.389Kg); $W_C$: Weight of the chain; $m_C$: Mass of the chain (0.341Kg); $g$: gravitational constant (9.81m.s$^{-2}$); $T_c$: Tension applied on the chain; $M_B$: Bending moment applied on B; $M_C$: Bending moment applied on C; $VBM_A$: Resulting ventral bending moment around the axis of rotation A.

**Statistical Analysis**

To compare the 3 stabilization techniques, the distribution of each continuous dataset was tested for normality prior to statistical testing. Normality was evaluated using a Shapiro-Wilk test and examination of the residuals. If the data was not normally distributed, normalization was attempted via logarithmic transformation. Normally
distributed datasets were tested using one-way analysis of variance (ANOVA), while non-normally distributed datasets were analyzed using Kruskal Wallis test (non-parametric one-way ANOVA). Categorical variables describing technical outcome were numerically graded and treated as continuous variables. For instance, mean number of dangerous, hazardous and optimal screws were compared between groups. Alignment was graded 0 (appropriate) or 1 (inappropriate) while apposition was graded 0 (optimal), 1 (suboptimal) or 2 (poor) to allow statistical comparison. To increase the statistical power, MI5 and MI6 were also pooled together to compare technical outcome between PMMA cemented constructs and TSF.

To compare the safety profile of the different implantation sites the percentage of dangerous, hazardous and optimal screws were compared between sites using a logistic regression model. Right sided and left sided implants were considered as same site for this analysis.

For the assessment of ventral bending moment at failure, a “survival” analysis of the stabilization constructs was also conducted to allow censoring of cases where the jig failed before the construct. This was achieved by considering instrumentation or bone failure as a “death” event while jig failure was equivalent to an “early withdrawal” of the experiment.

Statistical analysis of the data was performed using statistical software (SAS OnlineDoc® version 9.2, 2007, SAS Institute Inc. Cary, NC, USA). Statistical significance was set at 0.05.
III. Results

Technical outcome of ventral AA stabilization

Stabilization of all AA joints, including surgical approach and drilling of implant sites, was achieved successfully without any significant incident noted. All implants were placed as planned except for screws positioned in the C2 cranial articular surface. Many of these screws protruded slightly more than anticipated from the cis-cortex because the intended 2mm protrusion beyond the trans-cortex could not be obtained (dogs 3, 4, 5, 6, 11 and 13). This was due to the C1 caudal articular surface blocking the extremity of the screw. Furthermore, in dogs 11 and 13, this ventral protrusion occurred beyond midline and the contralateral screw could not be positioned as its predrilled direction was blocked by the first. This situation was overcome by placing a slightly shorter screw on one side to allow placement of the second screw.

CT images were obtained as planned in all cases. The raw data summarizing the postoperative CT findings is presented in Table III.2. Examples of different types of suboptimal technical outcomes are presented in Fig III.7. Statistical comparison of the 3 studied techniques revealed TSF achieved significantly better apposition than cemented constructs (p=0.0159). This statement was statistically significant when comparing TSF to MI6 (p=0.0086), however only a statistical trend was observed when comparing TSF to MI5 (p=0.0614). All other parameters of technical outcome including the quality of screw positioning and AA alignment were not statistically different between groups. There was only 1 screw identified with questionable cement embedding. Therefore statistical analysis could not be performed for this parameter. All calculated means, standard deviations and p-values for intergroup comparisons are reported in Table III.3.
Table III.2 Raw data of ventral AA stabilization technical outcome assessed using postoperative CT images.

M: Male; F: Female; L: left; R: Right; Cd VB: Caudal vertebral body; Cr Art Surf: Cranial articular surface; TrA: Transarticular; Alignment grading: 0=optimal, 1=suboptimal.

Apposition grading: 0=optimal; 1=suboptimal; 2=poor.

✔️: optimal outcome; ✘: suboptimal or poor outcome.

Green cell: TSF; Blue cell: MI5; Red cell: MI6.

<table>
<thead>
<tr>
<th>Random #</th>
<th>Random Group</th>
<th>Sex</th>
<th>Dangerous screws</th>
<th>Hazardous screws</th>
<th>Questionably cemented screw</th>
<th>Alignment</th>
<th>Apposition</th>
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<td>3</td>
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</tr>
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<td>✔️ 0</td>
</tr>
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<td>MI5</td>
<td>MI6</td>
<td>TSF vs MI5</td>
<td>TSF vs MI6</td>
<td>MI5 vs MI6</td>
<td>MI vs TSF</td>
</tr>
<tr>
<td>---------------------------</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>------------</td>
<td>------------</td>
<td>------------</td>
<td>-----------</td>
</tr>
<tr>
<td>Dangerous screw (# of)</td>
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<td>0.00</td>
<td>0.29</td>
<td>0.49</td>
<td>0.30</td>
<td>0.49</td>
<td>0.30</td>
</tr>
<tr>
<td>Hazardous screw (# of)</td>
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<td>0.49</td>
<td>0.71</td>
<td>0.95</td>
<td>0.39</td>
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<td>Alignment score</td>
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<td>0.00</td>
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<td>0.95</td>
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Table III.3  Statistical comparison of technical outcome in TSF, MI5 and MI6 techniques. Olive green cells: TSF; Blue cells: MI5; Red cells: MI6; Green p-values: Statistically significant; Orange p-values: Statistical trend; I: Non-normally distributed dataset.
Fig III.7 Anatomical and CT images illustrating different parameters of technical outcome and different types of failure observed during biomechanical testing (previous page).

(A, B, C) Dangerous screws; (D, E) Hazardous screws; (F,G) Apposition assessment. (Green arrows) Inappropriate implant position; (Red lines) Fracture lines at site of construct failure; (Yellow double-arrows) Sagittal vertebral canal diameter at AA junction; (Yellow circle) Vertebral canal area at AA junction. (A) C2 caudal vertebral body implant significantly violating the vertebral canal. (B) C2 cranial articular surface implant significantly violating the vertebral canal. Note that failure in this case occurred at the level of the C1 dorsal arch where the hooking system of the jig was positioned. (C) C1 pedicular screw significantly violating the vertebral canal. Failure of the cement at the level of the pedicular screws was commonly observed upon biomechanical testing of cemented constructs. (D) Minor vertebral canal violation of a C2 caudal vertebral body implant. (E) Left transarticular screw violating the atlanto-occipital joint. Compare to appropriate positioning of the contralateral implant. A bone fracture is present along C2 cranial articular surface which was observed in all TSF samples after ventral bending. (F) Optimal apposition obtained in a dog from TSF group. (G) Poor apposition observed in an AA joint stabilized with MI5 technique.


The quality of screw positioning was also compared between screw sites. Overall, 91 screws were placed, with 4 in a dangerous position (4.4%), 8 in a hazardous position (8.8%) and 79 in optimal positions (86.8%). The detailed distribution of screw positioning between the different screw sites is presented in Table III.4. C2 pedicular screws were the most successfully placed with an optimal position observed in all 14 implants. The site that revealed to be the most challenging was the monocortical screw placed in the C2 caudal vertebral body with 14.3% dangerous, 42.9% hazardous and only 42.9% optimal screws. When performing logistic regression of the proportion of optimal implants comparing this particular screw site to all other sites, it was found to be statistically different from C1 pedicular screws (p=0.0365, odds ratio= 0.1), C2 cranial articular surface screws (p=0.0365, odds ratio=0.1) and C2 pedicular screws (p=0.0117,
odds ratio=0.06) but not from C1-C2 transarticular screws (p=0.1285). The comparison of all other screw sites in between themselves did not reveal any statistically significant difference in quality of implant positioning.

### Table III.4 Distribution of the quality of screw placement across screw sites and comparison of the different sites through logistic regression analysis.

<table>
<thead>
<tr>
<th>Screw site</th>
<th>Dangerous</th>
<th>Hazardous</th>
<th>Optimal</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(# of)</td>
<td>(%)</td>
<td>(# of)</td>
<td>(%)</td>
</tr>
<tr>
<td>C1 Pdr</td>
<td>3</td>
<td>10.7</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>C1C2 TrA</td>
<td>0</td>
<td>0.0</td>
<td>2</td>
<td>14.3</td>
</tr>
<tr>
<td>C2 Cr Art Surf</td>
<td>0</td>
<td>0.0</td>
<td>3</td>
<td>10.7</td>
</tr>
<tr>
<td>C2 Pdr</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>C2 Cd VB</td>
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<td>3</td>
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<td>All sites</td>
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<td>4.4</td>
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<td>8.8</td>
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<table>
<thead>
<tr>
<th>Screw site</th>
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<th>p-value</th>
<th>O Ratio</th>
<th>Logistic Regression</th>
<th>p-value</th>
<th>O Ratio</th>
<th>Logistic Regression</th>
<th>p-value</th>
<th>O Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1 Pdr vs C1C2 TrA</td>
<td>0.2101</td>
<td>n/a</td>
<td>0.1959</td>
<td>C1 Pdr vs C2 Cd V</td>
<td>0.1192</td>
<td>n/a</td>
<td>0.0107</td>
<td>0.0365</td>
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<tr>
<td>C1C2 TrA vs C2 Pdr</td>
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<td>0.0158</td>
<td>5.81</td>
<td>C2 TrA vs C2 Cd V</td>
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<td>20.68</td>
<td>0.0365</td>
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<tr>
<td>C2 Cr Art Surf vs C2 Pdr</td>
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<td>5.81</td>
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<td>C2 Pdr vs C2 Cd V</td>
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<td>10.27</td>
<td>0.0117</td>
<td>0.0526</td>
<td>0.0117</td>
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</table>

Table III.4 *Distribution of the quality of screw placement across screw sites and comparison of the different sites through logistic regression analysis.*

Pdr: Pedicular; TrA: Transarticular; Cr Art Surf: Cranial articular surface; Cd VB: Caudal vertebral body; O Ratio: odds ratio; Green p-values/O Ratio: Statistically significant; Orange p-value/O Ratio: Statistical trend; n/a: not applicable.

### Biomechanical testing of ventral AA stabilization constructs

The stabilized AA joints extracted from the Beagle cadavers were all successfully tested under ventral bending. Load/displacement curves could be generated for each experiment using the raw data measured by the material testing system and pre-established equations (Fig III.8). The numerical data of these curves could be used to calculate ventral bending moment at failure, bending angle at failure and stiffness values in all cases (Table III.5).
Fig III.8 Load/displacement curves generated via loading of AA stabilization constructs in ventral bending.

Green curves: TSF group; Blue curves: MI5 group; Red curves: MI6 group; X-axis: Angle of bending; Y-axis: Ventral bending moment (VBM).
Table III.5 Raw data obtained from analysis of the load/displacement curves used for statistical intergroup comparisons of AA constructs biomechanical properties.

Green cell: TSF; Blue cell: MI5; Red cell: MI6.

A point of failure could be determined in all cases; however the type of failure could not always be identified by inspection of the sample. For instance if the 5% drop in ventral bending moment value was not clearly identified during testing, the load was occasionally increased beyond the first failure point until a second failure event occurred.
In those situations, the origin of the first failure point was uncertain. The common types of observed construct failure are depicted in Fig III.7. A bone fracture at the level of C2 cranial articular surface was observed in all TSF samples. In MI5 constructs, observed failure types included 4 instrumentations (57.1%), 1 jig (14.3%) and 2 unknown (28.6%). Similarly, MI6 failure types included 3 instrumentations (42.9%), 2 jig (28.6%) and 2 unknown (28.6%). All cases of instrumentation failure were due to cement fracture located at the level of the C1 pedicular screws except for dog 21 where both C1 pedicular screws pulled out of the lateral masses instead. Cases of jig failure were all due to a fracture of the C1 dorsal arch where the C1 hooking system was located. In dog 6, the tip of 1 hook also broke at the same time. Jig failure was only observed in MI5 and MI6 groups at higher values of ventral bending (7.37, 9.47 and 9.48 Nm).

The main anticipated confounding factor of the AA biomechanical comparisons was considered to be the vertebral size. This was assessed by measuring the body weight of each cadaver and the length of the C2 vertebral body. Statistical comparison of these 2 parameters did not reveal any significant difference between the 3 groups (Table III.6).

Table III.6 Statistical comparison of biomechanical properties in TSF, MI5 and MI6 stabilization constructs.

<table>
<thead>
<tr>
<th>Variable</th>
<th>TSF Mean ±SD</th>
<th>MI5 Mean ±SD</th>
<th>MI6 Mean ±SD</th>
<th>TSF vs MI5 p-value</th>
<th>TSF vs MI6 p-value</th>
<th>MI5 vs MI6 p-value</th>
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</thead>
<tbody>
<tr>
<td>Body weight (Kg)</td>
<td>9.06 ±1.49</td>
<td>8.21 ±0.93</td>
<td>8.31 ±1.40</td>
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<td>0.2979</td>
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<tr>
<td>L2 (cm)</td>
<td>2.05 ±0.08</td>
<td>2.00 ±0.19</td>
<td>2.14 ±0.15</td>
<td>0.4794</td>
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<td>0.0877</td>
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<tr>
<td>Stiffness at 0-5° (N.m/°)</td>
<td>0.19 ±0.05</td>
<td>0.20 ±0.08</td>
<td>0.25 ±0.09</td>
<td>0.9994*</td>
<td>0.3374*</td>
<td>0.3217*</td>
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<tr>
<td>Stiffness at 10-15° (N.m/°)</td>
<td>0.09 ±0.03</td>
<td>0.16 ±0.05</td>
<td>0.22 ±0.11</td>
<td>0.0295*</td>
<td>0.0011*</td>
<td>0.3107*</td>
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<tr>
<td>Stiffness ratio</td>
<td>2.32 ±0.80</td>
<td>1.28 ±0.36</td>
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<td>Angle at failure (°)</td>
<td>38.02 ±15.07</td>
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<td>VBM at failure (N.m)</td>
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<td>8.51 ±1.96</td>
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<td>0.7232</td>
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<td>Survival analysis</td>
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<tr>
<td>VBM at failure (N.m) with censoring</td>
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<td>0.0007</td>
<td>14.71</td>
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Olive green cells: TSF; Blue cells: MI5; Red cells: MI6; Green p-values/H Ratio: Statistically significant; *: Normalized dataset via logarithmic transformation; SD: Standard deviation; SE: Standard error; H Ratio: Hazard ratio.
The biomechanical characteristics of MI5 and MI6 were revealed to be very similar including stiffness values, stiffness ratio, bending angle at failure and ventral bending moments at failure. None of the biomechanical comparisons conducted between these 2 groups reached statistical significance (Table III.6).

The stiffness calculated in the 0-5° region was not significantly different between TSF (0.19±0.05Nm/°), MI5 (0.20±0.08Nm/°) and MI6 (0.25 ±0.09Nm/°). However, the same comparison conducted in the 10-15° region of the load/displacement curves revealed significant differences between TSF/MI5 (p=0.0295), and TSF/MI6 (p=0.0011). Analysis of the stiffness ratios was consistent with this result, TSF group (2.32±0.80) being significantly different from MI5 (1.28±0.36; p=0.007) and MI6 (1.25±0.41; p=0.0054). The distribution of stiffness and stiffness ratio data is presented in Fig III.9.

![Fig III.9 Box plots representing the 4 quartiles of stiffness and stiffness ratio values for TSF, MI5 and MI6.](image)

The angle of bending at failure was not significantly different between groups. In contrast, ventral bending moment at failure was significantly lower in the TSF group (3.66±1.23Nm) compared to MI5 (8.51±1.96Nm; p=0.0004) and MI6 (7.73±2.31Nm; p=0.0021) when using the raw data (Fig III.10). The same conclusions could be reached when conducting survival analysis with censoring of bending moment values where jig
failure was observed instead of a true construct failure. This statistical analysis revealed increased probability of failure for the TSF constructs compared to MI5 (p=0.0007; Hazard ratio=14.71) and MI6 (p=0.0007; Hazard ratio=14.93).

Fig III.10 Boxplots representing the 4 quartiles of ventral bending moments (VBM) at failure and Kaplan-Meier curves in TSF, MI5 and MI6. The Kaplan-Meier curve is applied here in an atypical context where “survival” of stabilization constructs are considered in relation to increasing values of VBM.
IV. Discussion

To the authors’ knowledge, this study constitutes the first in-vitro comparison of several canine ventral AA stabilization techniques. The primary purpose of this experiment was to describe and compare biomechanical properties of TSF, MI5 and MI6 techniques. Our data strongly suggested that TSF is markedly weaker than MI5 and MI6 constructs when submitted to ventral bending. Bending moment at failure was approximately 2 times larger in MI5 (8.51±1.96Nm) and MI6 (7.73±2.31Nm) compared to TSF (3.66±1.23Nm). This result implied that TSF constructs would be more prone to failure than PMMA cemented constructs, supporting anecdotal statements found in the literature (Sharp & Wheeler 2005). One limitation encountered during testing was the presence of jig failure occasionally occurring before the implants, PMMA cement or vertebrae failed. Developing a jig that would not interact with any part of the stabilization construct proved to be challenging as the entire ventral surfaces of C1 and C2 were covered with PMMA cement in the MI5 and MI6 groups. At the time of our study design there was no other report available in the literature which prompted us to manufacture the custom hooking systems as described in our materials and methods. Since then another biomechanical study overcame the problem by placing molding clay around the cemented construct to avoid any interaction between the potting structure and cemented construct (Kopf 2013). Even though we reached the physical limits of our jig in a few cases, this did not significantly impair our interpretation of the data. Indeed, these failure points could be considered as minimum values of bending moment at failure. And the fact that these events only occurred in MI5 and MI6 groups at higher values of bending moments, would logically imply that TSF would remain the weakest construct regardless. Furthermore, we were able to conduct a statistical survival analysis censoring the cases where jig failure occurred, which reached similar conclusions as with simple comparison.
of the means. This second type of analysis also provided hazard ratio values suggesting TSF was approximately 15 times more likely to fail than MI5 and MI6 constructs when submitted to values of ventral bending moment ranging from 0 to 12 Nm. This hazard ratio clearly illustrates the difference between TSF and the other 2 groups in our experiment, but this observation cannot be directly transposed to clinical conditions. Indeed, expected values of ventral bending moment in a Beagle dog would likely be much less than 12Nm under postoperative conditions where activity restriction is typically advised. Based on weight and length measurements taken on 10 Beagle cadaver heads, values of physiological ventral bending moment applied by the head on the AA joint can be estimated around 0.66±0.20Nm (see Appendix V). Therefore, despite the fact that TSF was experimentally identified as a weaker construct, prospective clinical data would be necessary to determine if this difference is clinically significant under physiological loads. Alternatively, cyclic fatigue load testing could be conducted experimentally to further compare the 3 constructs. Nevertheless, our results suggest that compared to TSF, cemented constructs would probably better tolerate uncontrolled high impact activity such as jumps or sudden ventral flexion of the head, resulting in transient increases in ventral bending loads.

Interestingly, TSF constructs consistently failed secondary to a bone fracture located on the C2 cranial articular surface in close proximity to the transarticular screws. This would imply that excessive fulcrum effect is applied in that region and that increased strength of the construct may be achieved with better distribution of the forces. For instance, if transarticular screws were left protruding ventrally and embedded in a small amount of PMMA, the construct could prove to be more resistant to ventral bending. Further biomechanical testing would be necessary to support that statement but proper distribution of forces is an important consideration when designing implants and stabilization constructs.
The construct stiffness is another important biomechanical characteristic when assessing stabilization techniques. Graphically, it corresponds to the slope of the load/displacement curve and is a measure of the motor unit resistance to the applied motion (Panjabi 1992a, Panjabi 1992b). Therefore, appropriate stiffness is necessary to achieve stability compatible with the bony fusion that is the ultimate goal in canine AA stabilization. When comparing the stiffness of TSF, MI5 and MI6 in the 0-5° region of bending which roughly corresponded to estimated physiological values of ventral bending loads, no statistical differences were identified between groups. This result would imply that all 3 methods provided similar stability under physiological loads and could theoretically achieve similar rates of AA fusion. Of course other parameters such as the possibility of placing a bone graft could have an impact on fusion rates (Festugatto, Mazzanti, et al. 2013). It could for instance be argued that TSF is more amenable to bone grafting given that the ventral bone surface is not covered by cement allowing hypaxial muscle to provide external vascularization. Such considerations would need to be investigated in a clinical setting to clarify the relative importance of different variables affecting AA fusion rates.

One limitation of our biomechanical testing method was the fact that we only tested ventral bending, while the AA joint is an extremely complex anatomical structure allowing wide multi-directional range of motion of the head (Watson, Evans, et al. 1986, Evans & de Lahunta 2012). However, similar designs have been reported in previous canine AA biomechanical studies as it is generally considered that the ventrally directed weight of the head is the most significant source of strain on the AA joint (Kopf 2013, Reber, Burki, et al. 2013). Due to the forward position of the head’s center of gravity in dogs, the main type of applied force on the cervical spine was considered to be ventral bending moment, although some component of dorsoventral shear can also be expected.
Another typical recommendation when performing biomechanical testing is to assess bone mineral density of the samples (Hicks, Pitts, et al. 2009, Hettlich, Allen, et al. 2013). This factor was not primarily taken into consideration in our study; however the randomization of the sample should theoretically have homogenized the 3 groups for any unassessed confounding factors.

A relatively unexpected finding in our study was the observed values of angle at failure varying from 17.6° to 62.2°. Such important deformations would either suggest marked elasticity of the construct or that partial failure such as individual screw deformation occurred before complete failure was obtained. In the TSF group there was some evidence that failure may have occurred before the 5% drop in bending moment was detected. Stiffness analysis in the 10-15° region of bending revealed a significant drop in stiffness of the construct with increased bending. Indeed, the initial stiffness in the TSF group was 0.19±0.05Nm/° which was not significantly different from MI5 and MI6, but this value decreased to 0.09±0.03Nm/° in the 10-15° region of bending. The mean stiffness ratio between 0-5° and 10-15° region of bending was 2.32 for TSF while it was 1.28 for MI5 and 1.25 for MI6. All of these values indicated that partial failure of TSF likely occurred earlier than detected with our definition of failure. The clinical importance of this limitation in our methodology is minimal given that our general conclusion stating that TSF is weaker than cemented constructs would remain valid.

Probably the most significant limitation of our study is the fact that we used a population of Beagle cadavers with a mean body weight of 8.53Kg, while a mean body weight of 3.6Kg was recently reported for dogs suffering from AA instability (Plessas & Volk 2014). The reason why Beagles were chosen for this study was that we did not have access to smaller sized cadavers and Beagle dogs were the closest experimental model we could obtain. Similarly, the growth stage of the dogs may have significant
implications when applying our results to the clinical setting. Indeed, a recent review highlighted the fact that 52-70% of canine AA instability cases are presented before 1 year old (Plessas & Volk 2014). Therefore clinicians should remember that different overall elasticity of the construct and different quality of screw anchoring may result in different types of construct failure in younger and/or smaller population of dogs. For instance, ventral loading resulted in screws pulling out of the bone for only 1 case in our experiment but such an event could be more common when using shorter screws in smaller/softer vertebrae.

A secondary goal of this study was to describe the safety profile of ventral AA stabilization. More precisely, we wanted to validate previously established definitions of optimal implant placement by studying the success rate of implant positioning using a method purely based on these definitions. This is why a 3D drill guide was used to position implants as closely as possible to the theoretical definitions. Overall, the positioning of screws in C1 and C2 proved relatively successful in the Beagle model with 86.7% of implants optimally positioned and only 4.4% of screws significantly violating the vertebral canal. This result suggested that satisfactory positioning of AA stabilizing implants can be achieved using the previously described definition of optimal implant placement in the Beagle model. These numbers are relatively similar to the rate of implant misplacement (4.4-7%) reported in a recent clinical meta-analysis comparing 2 AA fusion techniques in humans (Elliott, Tanweer, et al. 2012). However, in that human study only 0.25-0.34% of misplacements were considered clinically significant whereas we identified 4.4% dangerous screws in our experiment. Even if the described method of implant placement could be further improved, our data suggest that precise definition of optimal implant positioning can result in a relatively low risk of iatrogenic spinal cord injury during AA ventral stabilization. This statement is also supported by a recent retrospective clinical study reporting a positive outcome in 94% of dogs after ventral AA

The actual impact of our 3D drill guide prototype on accuracy of implant placement is unknown at this time. This study proved the drill guide could be used with reasonable success, but a prospective study comparing its accuracy to subjective visual estimations or advanced neuronavigation systems would be necessary to determine its benefits and limitations. Based on the fact that the position of the drill guide itself was based on visual recognition of the craniocaudal axis and sagittal plane, it can be expected that it would not achieve similar accuracy compared to advanced neuronavigation systems. Even if the current prototype could be used successfully in Beagle cadavers, we anticipate that miniaturization would be required to apply it in breeds typically affected with AA instability such as Yorkshire terriers or Chihuahuas.

Another purpose of our experiment was to compare the safety profiles of the 3 studied ventral AA stabilization techniques. Statistical comparisons of the parameters describing accuracy of implant placement did not reveal significant differences between TSF, MI5 and MI6 groups. This analysis was pursued further by comparing different implant sites rather than different techniques. The data revealed that the monocortical screw placed in C2 caudal vertebral body procured a lower rate of optimal positioning (42.9%) compared to any other sites (85.7-100%). This difference was statistically significant when comparing this particular site to all others except transarticular screws. However, it should be emphasized that our study was probably underpowered to conduct such comparisons. Our sample size was indeed primarily selected to detect differences in continuous variables (angles, bending moments, and stiffness) between 3 groups (TSF, MI5 and MI6). A significantly larger sample size would be anticipated when comparing categorical variables (such as quality of screw position) between 5 implant sites. This likely explains why transarticular screws achieving 85.7% of optimal
placement was not found statistically different from the C2 caudal vertebral body implants achieving only 42.9% optimal placement. Even so, the fact that this screw site was found significantly more prone to vertebral canal violation than some other screw sites strongly indicates that further intraoperative precautions should be taken when monocortical position is intended. Placement of monocortical screws is known to be technically challenging, with 9.7% vertebral canal violation reported in one cadaver study (Hettlich, Allen, et al. 2013). Based on that result, the authors recommended that drill stop systems should be used when placing monocortical implants. Despite using a drill guide providing control of the drilling depth, our results still seemed very modest compared to this report. It is important to note that our method of surgical planning was based on the analysis of previously obtained 3D data in a sample of 9 Beagles. Individualized preoperative measurements were not performed in any case. This methodology may have biased the results disadvantaging monocortical screws where the quality of implant positioning relies heavily on precise implant length, while bicortical implants mostly rely on accuracy of the insertion point and 3D direction. This could have participated in the lower rates of optimal position for the C2 caudal vertebral body implants compared to other screw sites. This interpretation was also supported by the fact that only 1 dangerous monocortical screw was identified and occurred in the dog with the smallest C2 vertebral body length (1.7cm) in our sampled population.

The main clinical implication of these findings is that precise individualized measurements of screw length as well as precise control of drilling depth are strongly recommended to place vertebral monocortical implants. Specific drilling depth criteria may need to be determined to improve the rate of vertebral canal violation associated with these implants. We subjectively elected to use 75% of the previously reported mean optimal bone corridor length. Using shorter drilling depth such as 66% of the corridor length could be considered to increase safety margins. Further investigation would be
necessary to determine the most appropriate method given that excessively reducing the drilling depth may also result in inappropriate bone purchase, impairing the stability of the construct.

At the other end of the spectrum, C1 and C2 pedicular screws were optimally placed in 89.3% and 100% of implants respectively. This differed significantly from a recent safety study where 100% vertebral canal violation was noted in pedicular implants placed in cervical vertebrae 4 and 5 (C4 and C5) (Hettlich, Allen, et al. 2013). This marked difference between both studies could be attributed to anatomical disparities between the well-developed C1 lateral masses and the very thin C4/C5 pedicles. But such justification would likely be inaccurate for C2 pedicles which are anatomically similar to C4 and C5. Another possibility could be that optimal pedicular position in previous studies have been based on descriptive anatomical data with the intention of avoiding both vertebral canal and transverse foramen (Corlazzoli 2008). We used a similar definition in our experiment for C1 pedicles but not in C2 where violation of the vertebral foramen was judged inevitable in smaller sized dogs based on previously collected CT data. Therefore our definition of C2 pedicular optimal position allowed penetration of the transverse foramen, implying that wider safety margins were available laterally and possibly explaining our considerably higher success rate compared to previous studies. Nonetheless, surgeons should be aware that using such implant position almost inevitably results in vertebral artery injury which is of unknown clinical significance in dogs, especially if occurring bilaterally. An alternative method reported in the literature to prevent vertebral artery injury is to position implants in transverse processes rather than pedicles (Hicks, Pitts, et al. 2009). Even though this method proved safe and biomechanically viable in a population of larger dogs, we considered it unlikely to be applicable to dogs suffering from AA instability given that transverse processes in predisposed breeds are often extremely small.
Another important parameter of the technical outcome investigated in our study was the quality of C1 and C2 apposition after stabilization, with results showing a significantly better apposition in TSF group compared to MI5 and MI6. Intraoperative difficulties for the reduction of AA subluxation are well described in the literature (Platt, Chambers, et al. 2004, Sharp & Wheeler 2005, Forterre, Vizcaino Reves, et al. 2012, Dewey 2013). A tendency of C2 vertebral body to move craniodorsally with AA instability is typically reported, displacing C2 away from the surgeon when using a ventral approach. This renders anatomical reduction and implant positioning technically challenging. Several reduction methods have been reported in the literature to overcome this problem. Direct methods of reduction include the use of intra-articular retractors, orthopedic reduction forceps, or placing monocortical bone screws to apply caudoventral traction using suture material (Platt, Chambers, et al. 2004, Sharp & Wheeler 2005, Dewey 2013). An indirect method was also described using a Gelpi retractor placed between the base of the occipital bone (intercondyloid incisure) and the C2-3 intervertebral disc (fenestrated beforehand) to apply craniocaudal distraction (Forterre, Vizcaino Reves, et al. 2012). In our experiment, we used a direct method of reduction in the TSF group using towel clamps to appose C2 against C1 whereas an indirect method was used in MI5 and MI6 groups applying lateral muscular tension with Gelpi retractors. Both methods achieved successful intraoperative reduction of the AA joint when assessed visually and allowed placement of stabilization implants. However, on postoperative CT images several cases of inappropriate AA apposition were identified in the MI5 and MI6 groups. It can be hypothesized that this occurred either while positioning PMMA cement due to excessive pressure applied on the C2 vertebral body, or because Gelpi retractors may have been removed too early, before PMMA curing had occurred, allowing the vertebrae to return to their unreduced state. Evidence supporting this second hypothesis was identified in some cases where the imprint of hypaxial musculature was visible on the cemented
construct. This result implies that direct methods of reduction should be preferred if available. For instance, suture material attached to the heads of the 2 screws placed in the C2 cranial articular surface could be used to maintain ventral tension on C2 while PMMA cement is placed. A short portion of suture would therefore be incorporated in the cemented construct, but this would likely prevent the C2 dorsal subluxation observed in our study.
V. Conclusion

To summarize, this experiment provided strong evidence that TSF construct is markedly weaker than MI5 and M6 constructs when submitted to higher loads of ventral bending. This implies that cemented constructs would likely resist better than TSF to an acute increase in ventral bending loads such as generated by a sudden jump during the postoperative period. Further investigation would be necessary to determine if this finding could be extended to more physiological cyclic loads better representing constant/daily strains applied on the construct over weeks to months after surgical stabilization. Failure observed with the TSF technique suggested a fulcrum effect likely occurs at the level of the C2 cranial articular surface. The TSF technique could therefore be improved by developing a surgical method aiming at dissipating this fulcrum via better distribution of the applied forces. All 3 tested constructs provided similar stiffness under physiological loads of ventral bending, suggesting they could achieve similar rates of AA fusion from a biomechanical perspective.

From a safety perspective, it could be established that the monocortical implant used in MI5 techniques was significantly more prone to vertebral canal violation using our method. This could however likely be improved in practice using individualized preoperative planning including precise length measurements of the intended drilling site and drill stop systems. Implants used in TSF and MI6 constructs proved to be relatively safe to position away from the vertebral canal, although clinicians should be aware that MI6 screws placed in C2 pedicles almost inevitably will result in transverse foramen violation potentially causing bilateral vertebral artery injury. The clinical significance of such arterial disruption in dogs is unknown.

Finally, the TSF method achieved better reduction of the AA subluxation than cemented constructs in our experiment, likely due to the direct method of reduction
applied concomitantly to the placement of stabilizing implants. Based on that finding we would recommend using suture material to apply ventral traction on the screws placed in C2 cranial articular surfaces while placing PMMA cement or one of the previously described direct or indirect methods of AA reduction.
VI. References


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CHAPTER IV - GENERAL SUMMARY AND CONCLUSIONS

Summarized rationale

The research questions that eventually constituted the basis for the current thesis were originally formulated at the Ontario Veterinary College Health Science Centre after being confronted with some challenging clinical cases of canine AA instability. Several of our veterinary neurosurgeons had similar clinical experience carrying out these surgeries which highlighted their complexity and high technicality. Even experienced surgeons considered AA stabilization as unacceptably hazardous for the patient. Major suspected reasons for these clinical observations included the extremely small body size of these patients with restricted margins of error when positioning implants and poorly described anatomical landmarks providing little guidance to perform these procedures safely. This combination of technically demanding procedures performed in very small dogs with lack of precise theoretical description motivated us to conduct research in this area.

Another aspect of these surgeries that was necessary to consider to formulate clinical recommendations was the biomechanical properties of stabilization constructs. Some data from the literature suggested that ventral stabilization was superior to dorsal techniques but to our knowledge there was neither any study comparing different ventral constructs nor any significant information justifying the use of one technique over another.

Development and validation of a novel method of bone corridor analysis

Our initial objective was to provide data to the veterinary community describing as
precisely as possible optimized methods of AA stabilization. As we began to work on the
description of optimal implant positioning, we rapidly realized that a novel method of
analysis of the vertebrae would be necessary to precisely depict the complex
tridimensional (3D) interrelationships between anatomical structures and implants.
Consequently, a novel method of optimal safe implantation corridors (OSICs) computed
tomography (CT) analysis using OsiriX™ software was proposed. The major
advancement offered by this method was that it allowed the overcoming of limitations
encountered in previous studies due to subjective definitions of optimal implants. This
was achieved via 3D simplification of bone corridors into geometrical shapes with well-
defined optimal centered axes. The method heavily relied on the positioning of region of
interest (ROI) points on CT images. The major limitation associated with ROI point
placement is that OsiriX™ associates each ROI to a specific CT slice resulting in non-
continuous space. In other words, the space located between CT slices could not be
represented with ROI points. As a result, theoretical error in any ROI positioning up to
half the slice thickness (0.3125mm) could be expected in our study design. This was
regarded as likely having low impact on OSIC descriptions but this source of error could
have been minimized for instance by reformatting all CT studies to 0.1mm slice
thickness prior to ROI positioning.

In order to apply this method in a time-efficient manner, a mathematical model was
developed to semi-automatize the process. This involved relatively advanced 3D
modeling of the CT space involving multiple equations and 2 separate software
products. Therefore, it was elected to validate the method using a randomized
prospective study assessing agreement between calculated and manually measured
values on CT images and, also, the reproducibility of the 3D anatomical space modeling
based on predefined landmarks (O,A,B points). To do so, we hypothesized 1: that the
mathematical calculations would have high concordance with the manual measures, and 2: that relatively small error would be identified by the positioning of predefined landmarks on CT images. This study demonstrated excellent concordance between the semi-automated mathematical calculations and manually measured values ($\rho_c>0.998$) which validated our first hypothesis. Our data also implied that angles defining the 3D position of implants and safety margins could be accurately obtained using OsiriX™ measurement tools (<1.3° error with 95% probability), although this would be more time consuming compared to the semi-automated method. Similar concordance analysis, revealed that positioning of the landmarks used for anatomical space modeling induced low errors (overall 1.6°, up to 3.7° for some subsamples; with 95% probability). Such error values were considered small given that a minimum 15-20° bone corridor angular width would likely be necessary to recognize a corridor as acceptably safe. These results validated our second hypothesis, allowing us to conclude that the use of OsiriX™ with implementation of mathematical equations on exported 3D coordinates was an efficient and reproducible tool which could be applied to describe AA OSICs.

**Descriptive study of AA OSICs**

An exhaustive definition of optimal implant position should include anatomical landmarks localizing its insertion point, coordinates describing its direction in 3D space with respect to the targeted anatomical structure, and numerical measure of its length. This last parameter is particularly important for implants directed toward vital anatomical structures such as monocortical screws aimed toward the vertebral canal. In addition, the safety margins surrounding the optimal implant position can be assessed to provide insight on technical feasibility of optimal positioning, potential associated risk for the patient, and also to estimate optimal diameter of the implant. All of these parameters of
OSICs could be analyzed using our previously validated CT method in a population of 27 dogs. To our knowledge, this extensive descriptive dataset constitutes the first comprehensive analysis of ventral AA OSICs in dogs. A secondary objective for that study was to compare OSICs characteristics between dogs suffering from AA instability (AAI), normal Toy breed (N-Toy) dogs and normal Beagle (N-Beagle) dogs to determine if the latter 2 groups could constitute an acceptable geometrical model of AAI dogs. The statistical hypothesis formulated for that purpose was that all 3 groups would have similar OSIC characteristics except for dimensions correlated to the dog’s body size (length and width).

Probably one of the most significant results of this study was the determination of objective definitions for optimal insertion points based on ventral anatomical landmarks that were revealed to be similar in all 3 groups. The diagrams presented in chapter II were designed to be used as intraoperative visual guidelines. Similar schematics and tables were also provided to summarize the data describing the optimal 3D direction of each implant defined by 2 projected angles. Statistical comparison of projected angles values between the 3 groups revealed that most implant sites had similar projected angles with differences in mean values typically inferior to 5°. These observations validated our statistical hypothesis demonstrating that all 3 groups had similar geometrical distribution of AA OSICs. However, significant individual variation was identified between dogs in all groups with a range of values between 20-30° for most implant sites. This finding suggested that individualized surgical planning would likely optimize accuracy compared to using the mean or median of reported values.

The analysis of safety parameters such as critical safety angles and critical technical angles representing respectively the minimal margins of error for vertebral canal violation and OSIC angular widths also provided pertinent clinical information. This data
was summarized in chapter II using tables and diagrams. The calculation of safety angles based on different implant diameters suggested that 1.5mm should not be exceeded in most Toy breed dogs except when placed in wider bone corridors such as the C2 cranial articular surface or C2 pedicles. Surprisingly, intergroup comparison also revealed that Beagle lateral masses were proportionally narrower with similar width and safety angle values to those observed in Toy breeds. Overall, when comparing the data describing safety margins for each implant to the observed individual variations in optimal 3D position, some implants would be considered particularly hazardous if positioned based on mean or median projected angles. This was particularly striking for the C1-C2 transarticular implant which had a very narrow critical safety margin and therefore would benefit most from individualized surgical planning. Alternatively, the use of well-defined anatomical landmarks might also overcome this problem, for instance using the alar notch as a targeted exit point rather than projected angle values.

**Application of the reported AA OSIC definitions in a cadaver model**

Once we established objective definitions of the most commonly used implant sites we elected to conduct a prospective randomized study comparing 3 techniques of AA stabilization in a population of 21 Beagle cadavers. The 3 techniques included transarticular screw fixation (TSF), and polymethylmethacrylate (PMMA) cemented constructs with either 5 or 6 screws (MI5 or MI6). One of the objectives for this study was to determine whether the previously described definitions of optimal implant placement could be used successfully in situ. To achieve this goal, we designed a 3D drill guide prototype allowing us to reproduce intraoperatively our definitions of optimal implant placement. The statistical hypothesis used for that part of the study was that all 3 techniques would result in a similar technical outcome and all implant sites used would
be placed with similar accuracy. Technical outcome included quality of implant positioning, AA alignment and AA apposition after stabilization.

Out of 91 screws placed, 4 were considered dangerous (4.4%), 8 hazardous (8.8%) and 79 optimal (86.8%) positions. This result suggested that the previously described definition of OSICs could be used with satisfactory accuracy. Intergroup comparison revealed that the TSF technique achieved excellent apposition compared to MI5 and MI6 where several cases of unsatisfactory apposition were observed. Based on this finding, we recommended applying ventral tension on the C2 vertebral body while PMMA is placed to insure apposition is maintained during curing. The comparison of different screw sites revealed that monocortical screws placed in the C2 caudal vertebral body was more prone to vertebral canal violation than other sites. This finding highlighted the importance of precise positioning of monocortical screws which would be likely optimized using individualized planning and drill stop devices.

**Biomechanical comparison of 3 AA ventral stabilization techniques**

Finally, we wanted to compare biomechanical characteristics of the 3 studied stabilization constructs. This was achieved by measuring tension loads while applying ventral bending to each construct until failure was observed. The statistical hypothesis was that all 3 groups would have similar mean values of stiffness under physiological loads and similar ventral bending moment at failure. Biomechanical testing required the manufacturing of a custom jig to position the constructs in the material testing machine.

This analysis revealed that constructs’ stiffness under physiological loads were not statistically different between groups. From a biomechanical perspective this equates to stating that all 3 methods could provide similar AA fusion rates as long as failure does not occur and the microenvironment necessary for osteoproliferation is preserved. In
contrast, we observed an increased probability of failure for the TSF constructs compared to MI5 (p=0.0007; hazard ratio=14.71) and MI6 (p=0.0007; hazard ratio=14.93) under ventral bending. Failure occurred at higher values of bending, mostly representing an acute increase in load such as a jump or trauma to the head during the postoperative period. These results allowed us to reject the statistical hypothesis, proving that PMMA cemented constructs were markedly stronger than TSF against ventral bending in our Beagle model. Failure in the TSF group occurred via fracture of the C2 cranial articular surface, suggesting a fulcrum effect at this level. This could justify modifying the TSF technique using additional implants to better distribute the load along the C2 vertebral body.

**Future research perspectives**

Canine AAI represents a complex diagnostic and therapeutic challenge. Part of this complexity comes from the 3D interrelationships between C1 and C2. Proper planning of a reconstructive surgery typically implies realigning subluxated bones or fractured bone fragments. Performing this type of 3D anatomical realignment at the surgical planning stage would be extremely helpful to optimize the implant positioning for each individual patient. The mathematical model we used for simulation of the vertebral anatomical space could be used to automatize such anatomical correction. This would require acquiring a larger dataset to identify characteristics defining the AA joint in neutral position and developing advanced 3D software allowing repositioning of multiple 3D objects. Such software would also be helpful to compare different stabilization constructs such as plating systems in virtual 3D space.

Other technological advancements such as neuronavigation, 3D printing or bio-absorbable implants will likely revolutionize the way we currently think of surgical
planning and will likely dramatically improve the technical outcome of spinal instrumentation. However, this type of surgery remains relatively infrequently performed in veterinary medicine and wide access to these technologies may be delayed for decades. We therefore developed a simple, inexpensive 3D drill guide that allowed placing implants with satisfactory accuracy, although still relying on visual recognition of the sagittal plane and craniocaudal axis. This prototype would need to be miniaturized for Toy breed dogs and compared to un-guided implant placement to determine its actual value.

Further investigation of AA stabilization construct biomechanical properties would be necessary to consolidate our findings. The first objective would be to develop a jig that could be used in smaller sized vertebrae such as Toy breed AA joints. Even though the comparison between techniques would likely result in similar differences, it cannot be excluded that the type of failure may be different in clinically affected dogs who are often extremely small and immature dogs. The use of other bone models such as feline vertebrae or plastic 3D printed vertebrae may have to be considered to conduct experiments with larger sample sizes. Validating an easily accessible model would indeed be extremely helpful to be able to conduct multiple experimental comparisons of different constructs under different loading modalities. For instance, testing the constructs under physiologic cyclic loads may better represent constant/daily strains applied over weeks to months after surgical stabilization and result in different types of failure such as screw fractures.

Eventually, all of this experimental data would have to be validated with well-designed prospective randomized clinical trials. However, given the relatively uncommon nature of the disease, the selection of treatment protocols should be based on preclinical experimental data that is as extensive as possible in order to maximize the safety and
clinical value of studies conducted on actual patients.

General conclusions

To conclude, we believe this manuscript provides significant advancement in the understanding of AA 3D vertebral anatomy. Our results suggest that using precise definition of optimal implant positioning and individualized presurgical planning would likely result in satisfactory technical outcome using commonly reported AA ventral stabilization techniques. Reproducing these precise definitions intraoperatively remains a major technical challenge. We therefore proposed a tentative solution by designing a novel 3D drill guide. This proposition is certainly imperfect given that visual alignment of the guide is required intraoperatively, nevertheless it may reveal a viable inexpensive alternative to currently available advanced neuronavigation systems.

Specific technical recommendations were provided based on our results including the importance of maintaining appropriate reduction while PMMA is curing and the need to take particular precautions when placing monocortical implants. Finally, our biomechanical data suggested the TSF construct was markedly weaker than PMMA cemented constructs, which may be improved by better distributing the applied forces on the C2 vertebral body.
A.I.1. Generalities on determination of geometrical centers

To determine the center of complex 3D shapes, a specific plane of interest was identified in 3D-MPR mode. This sectioning method of the 3D shape would allow studying simple 2D geometrical objects such as triangles, parallelograms, trapezoids, irregular quadrilaterals, or ellipses. For the purpose of our study, the center of interest of these geometrical shapes could be defined as the point that is the most equidistant from the sides. Such position would theoretically maximize the diameter of the implant that could be inserted into the bone corridor. The method is detailed in Fig A.I.1.

Fig A.I.1  Diagrams representing the geometrical methods of determination of OSIC centers.
(A) For triangles and irregular quadrilaterals, the intersection of 2 bisectors was used to determine the center C. For parallel lines, a bisector line is defined as the equidistant parallel line. This geometrical principle could be used for parallelograms and the parallel lines of a trapezoid. The center of triangles and parallelograms could alternatively be determined using the inscribed circle, and the intersection of diagonals respectively. Centers of ellipses were provided by OsiriX™ ellipse ROI tool.
(B) In OsiriX™, the ROI points were associated to specific slices, while centers could be located in between. In these situations, 2 ROI points were placed cranially and caudally to delineate the center point as precisely as possible.
A.I.2. Determination of C1 pedicular optimal implantation axis

The OSIC of the C1 pedicular implants can be defined as the caudal region of C1 lateral masses. We elected to focus on the caudal region of the lateral masses because it is the broadest region and also to avoid penetrating the small artery branching from the vertebral artery toward the vertebral canal at the cranial extremity of the alar foramen. Because of the complexity of the lateral masses 3D structure, we elected to define the optimal position, focusing on its narrowest portion at the level of vertebral artery as well as C1 pedicular region as depicted in Fig A.I.2.

Fig A.I.2  Step-by-step method of determination of C1 pedicular optimal implant.
(A) 3D reconstruction showing C1 pedicle simplified into a prism, (B) Geometrical determination of the center of the ventral surface, (C) Geometrical determination of the center of the lateral surface.

Point 1 is first positioned in 3D-VR at the craniomedial extremity of the alar foramen (just caudal to the entry point of the tranverse vertebral artery branch). The dorsal plane passing through 1, is localized in MPR-mode. Point 2 (closest point to 1 on the inner cortical) and point 3 (caudomedial extremity) are then positioned. In the same plane, a line tangent to the caudal articular surface of C1 passing through 3 is drawn. The intersection between that line and the lateral cortex localize point 4. Point 5 is defined in 3D-VR as the caudodorsal extremity of the lateral mass. Point 6 is identify in 3D-MPR in the plane 1,4,5 to delineate the pedicle craniodorsal extremity. The centers 7 and 8 are geometrically determined in 3D-MPR. The line 7-8 define the optimal implant placement.
A.I.3. Determination of C1-C2 transarticular optimal implantation axis

The main challenge of defining C1-C2 transarticular implant position was that 2 separate vertebrae were involved. We elected to define the OSIC based on C1 lateral masses because it is located further away from the implant’s insertion point and contains the longest corridor length (A.I.3). These characteristics suggested C1 was more likely to restrict the optimal implant position compared to C2 cranial articular surface which is a very broad structure. The intent when simplifying the corridor was to describe the region of the lateral masses that would avoid both the vertebral canal and the alar foramen.

Fig A.I.3 Step-by-step method of determination of C1-C2 transarticular optimal implant. (A) 3D reconstruction showing C1 pedicle simplified into a pyramid, (B) Geometrical determination of the center of the pyramid base, (C) Optimal axis determined by centering its position dorsoventrally and lateromedially rotating around the center.

Point 1 is first positioned in 3D-VR at the medial portion of the alar notch. Point 2 is then positioned in 3D-VR mode by tracing a line passing through 1 and the medial border of the craniomedial border of the alar foramen. Point 2 is located at the intersection between this line and the ventral ridge of the caudal articular surface of C1. Point 3 is located in 3D-VR at the ventromedial extremity of the caudal articular surface of C1. Point 4 is placed in 3D-VR at the caudodorsal extremity of the same articular surface. Point 5 is placed using 3D-MPR in the plane 2,3,4 to delineate the laterodorsal extremity of pyramid. The center of the base 6 is then geometrically determined in 3D-MPR and the optimal implant placement is localized by rotating the axis around this center both dorsoventrally and lateromedially. Note that the positioning of the insertion point on C2 is somewhat imprecise due to slight misapposition between C1 and C2.
A.I.4. Determination of C2 cranial articular surface optimal implantation axis

The cranial articular surfaces of C2 can roughly be assimilated to 2 semi-ellipsoids oriented obliquely on each side of the cranial region of C2 vertebral body. The centered axis of a hemi-ellipsoid can be defined as the intersection of 2 orthogonal bisector planes. However, such theoretical centered axis would be oriented within the dorsal plane which cannot be achieved when placing implants in practice. Therefore, a subjective angle of $30^\circ$ from the dorsal plane was used to provide the description of an “optimal” implant that can be used in surgery (Fig A.I.4).

**Fig A.I.4**  Step-by-step method of determination of C2 cranial articular surface optimal implant.

(A) 3D reconstruction showing C2 cranial articular surface simplified into a semi-ellipsoid (in yellow), (B and C) Geometrical determination of the center (1) of the semi-ellipsoid using 2 ellipses placed in 2 orthogonal planes (black ellipses). (C) Optimal axis placed at $30^\circ$ from the dorsal through the center of the simplified corridor.
A.I.5. Determination of C2 pedicular optimal implantation axis

The method of geometrical simplification for C2 pedicles is similar to C1 pedicular OSIC. The main difference is that the vertebral artery cannot be avoided at this level due to extremely thin pedicles. As a result, the transverse foramen was included into the OSIC and optimal implants were knowingly passing through the foramen (Fig A.I.5).

Fig A.I.5  Step-by-step method of determination of C2 pedicular optimal implant. (A) 3D reconstruction showing C2 pedicle simplified into prism, (B and C) Positioning of ROI landmarks are initially performed in the transvers plane, (D and E) Geometrical determination of the ventral and lateral surfaces of the prism similar to C1 pedicle. All points are placed using 3D-MPR for this OSIC. Point 1,2 and 3 are first positioned in the transverse plane a the level of the cranial extremity of the transverse foramen. 1 is defined as the lateroventral margin of the pedicle, 2 is defined as the lateroventral margin of the vertebral foramen and 3 is defined as the dorsal margin of the pedicle identified by drawing a line tangent to the vertebral foramen passing through 2. Point 4 is placed similarly to 2 but at the level of the caudal extremity of the transverse foramen. Then, the plane 1,2,4 is identified in 3D-MPR and 5 is positioned to identify the laterocaudal limit of the ventral surface of the prism. Similarly, the plane 1,3,5 is localized and 6 is placed caudodorsally. The optimal implant is defined as the line passing through both ventral and lateral surface centers 7 and 8.
A.I.6. Determination of parasagittal C2 caudal vertebral body optimal implantation axis

The caudal vertebral body of C2 has a pyramidal shape naturally. However, the base located at the level of the epiphysis is concave due to intervertebral disc articulation. This slight irregularity was considered in the process of 3D simplification by defining the sagittal plane as a bisector plane of the corridor as depicted in Fig A.I.6.

Fig A.I.6  Step-by-step method of determination of C2 caudal vertebral body (parasagittal) optimal implant.
(A) 3D reconstruction showing C2 caudal vertebral body simplified into a pyramid with concave basis, (B) Geometrical determination of the center of the simplified corridor in sagittal plane. Point 1, 2 and 3 were placed in 3D-VR with 1 being the ventral eminence, and 2-3 the lateral extremities of C2 epiphysis. Point 4 was placed using 3D-MPR in the sagittal plane at the intersection between the ventral limit of the vertebral foramen and a line tangent to the ventral surface of C2 passing through 1. The point 5 was defined in 3D-MPR as the intersection between the 2,3,4 plane and the caudal extremity of C2 epiphysis sagitally. The center of the triangle 1,4,5 was then geometrically determined and the optimal axes defined as the line 6-2 for the right implant and 6-3 for the left.
A.I.7. Determination of sagittal C1 and C2 optimal implantation axis

The determination of optimal positions of implants located in the sagittal plane did not require 3D geometrical simplification as the optimal position was predefined within 2 dimensions. Instead an insertion point was subjectively defined for C1 ventral arch and C2 cranial vertebral body implants, while an exit point was predefined for C2 caudal vertebral body implants. This allowed positioning the optimal implant in space by determining the sagittal bisector of each corridor (Fig A.I.7).

Fig A.I.7  Step-by-step method of determination of C1 and C2 sagittal optimal implants. (A) 3D reconstruction showing the typical location of the 3 sagittal implants, (B) Geometrical determination of each optimal axis using the bisector method. For C1 ventral arch and C2 cranial vertebral body the angular width of the bone corridor was measured from the insertion point perspective, while the exit point was used for C2 caudal vertebral body. The bisector lines of these angles define the optimal implant positions.
APPENDIX II - DETAILED METHOD OF OSICS CT ANALYSIS: CARTESIAN VECTORIAL CALCULATIONS USED TO DETERMINE PROJECTED ANGLES OF OPTIMAL IMPLANTS

The following notations will be used:

\( \vec{X} \) : vector \( X \)

\( \| X \| \) : norm of vector \( X \)

\( \hat{X} \) : unit vector \( X \) (normalized)

\( (\cdot)_X \) or \( [\cdot]_X \) : coordinates of a point or vector defined with respect to a basis \( X \)

\( \theta_{\text{Anatomical plane}(\hat{X})} \) : projected angle on an anatomical plane with respect to the vector \( \hat{X} \)

A Cartesian 3D coordinate system is most commonly used to define spatial relationships in \( \mathbb{R}^3 \). A CT study provides 3D representation of a finite space which is the sum of all the slices of the anatomical region studied. The basis (or coordinate system) for the CT space is defined by 3 orthogonal unit vectors \( (\hat{i}, \hat{j}, \hat{k}) \) and an origin \( O \) \( (0,0,0)_{CT} \). By definition, the coordinates of the basis unit vectors are \( \hat{i} (1, 0, 0)_{CT} \), \( \hat{j} (0, 1, 0)_{CT} \), and \( \hat{k} (0, 0, 1)_{CT} \). The basis \( (\hat{i}, \hat{j}, \hat{k}) \) is then used to define any vector \( \vec{u} (x, y, z)_{CT} \) within the CT space as a linear combination of the basis vectors. This is represented by the equation \( \vec{u} = x \cdot \hat{i} + y \cdot \hat{j} + z \cdot \hat{k} \) ; which is basically the definition of coordinates relative to a basis. In our study, the coordinates from OsiriX™ are provided with respect to the CT basis which has little anatomical meaning. These coordinates can be used to calculate non oriented dimensional relationships (distances or absolute angles) but equations to define spatial relationships with respect to anatomical axes and planes (projected distances and angles) would be extremely complicated if used as is.

In order to define spatial relationships relative to anatomical structures (in our case the vertebrae C1 and C2), a change of coordinate systems is applied which greatly simplify the equations. An anatomical Cartesian coordinate system can be defined by 3 unit vectors \( (\hat{i}_1, \hat{j}_1, \hat{k}_1) \) and a subjective origin \( O_1 \) \( (0, 0, 0)_{C1} \) for C1 and similarly \( (\hat{i}_2, \hat{j}_2, \hat{k}_2) \) and \( O_2 \) \( (0, 0, 0)_{C2} \) for C2. These vectors are chosen so that \( \hat{i}_1 / \hat{i}_2 \) are
direction vectors of C1 and C2 craniocaudal axes, \( \vec{j}_1 / \vec{j}_2 \) are direction vectors of the ventrodorsal axes and \( \vec{k}_1 / \vec{k}_2 \) are direction vectors of the right to left axes. This will define 2 “right-handed” coordinate systems respectively for C1 and C2. In practice, this is achieved by selecting 3 points within the sagittal plane of the vertebra of interest.

For C1, the origin of the system \((O_1)\) is placed at the level of the ventral tuberosity, whereas the other 2 points \(A_1\) and \(B_1\) are placed along the craniocaudal axis. The ventrodorsal axis of C1 is defined as the line passing through the cranial margin of the dorsal and ventral arch in the sagittal plane. The craniocaudal axis is defined as its perpendicular in the same plane. For C2, the origin \((O_2)\) is placed in the sagittal plane, at the cranioventral eminence located at the base of the dens. The other 2 points \(A_2\) and \(B_2\) are placed along the craniocaudal axis defined as the sagittal ventral border of the vertebral foramen. The following equations will demonstrate how the change of coordinate system is operated on the CT system values in order to calculate projected angles relative to the anatomical planes of the vertebrae (sagittal, transverse and dorsal). C1 is used as example, but the equations are identical for C2.

**STEP 1 – Determination of coordinates of the C1 basis vectors in CT system**

To simplify the equations, the unit vectors’ coordinates with respect to the CT base will be noted:

\[
[\vec{i}_1]_{CT} = \begin{bmatrix} X_{\vec{i}_1} \\ Y_{\vec{i}_1} \\ Z_{\vec{i}_1} \end{bmatrix}, \quad [\vec{j}_1]_{CT} = \begin{bmatrix} X_{\vec{j}_1} \\ Y_{\vec{j}_1} \\ Z_{\vec{j}_1} \end{bmatrix}\quad \text{and}\quad [\vec{k}_1]_{CT} = \begin{bmatrix} X_{\vec{k}_1} \\ Y_{\vec{k}_1} \\ Z_{\vec{k}_1} \end{bmatrix}
\]

By definition, \(\overrightarrow{A_1B_1}\) has the same direction as \(\vec{i}_1\) and therefore \([\vec{i}_1]_{CT}\) can be directly calculated from the following equation:

\[
[\vec{i}_1]_{CT} = \frac{[\overrightarrow{A_1B_1}]_{CT}}{||\overrightarrow{A_1B_1}||} = \frac{1}{\sqrt{(x_{B_1} - x_{A_1})^2 + (y_{B_1} - y_{A_1})^2 + (z_{B_1} - z_{A_1})^2}} \begin{bmatrix} x_{B_1} - x_{A_1} \\ y_{B_1} - y_{A_1} \\ z_{B_1} - z_{A_1} \end{bmatrix}_{CT}
\]
In other words $X_{\kappa_1}, Y_{\kappa_1}$ and $Z_{\kappa_1}$ can be directly calculated from OsiriX $A_1$ and $B_1$ coordinates.

$\vec{k}_1$ is the normal vector of the sagittal plane (i.e. right to left axis is perpendicular to the sagittal plane). The cross product $\vec{\kappa}_1 \times \vec{O_1A_1}$ can therefore be used to determine $[\vec{k}_1]_{CT}$.

$$[\vec{k}_1]_{CT} = \left[ \vec{\kappa}_1 \times \vec{O_1A_1} \right]_{CT} = \frac{[\vec{\kappa}_1 \times \vec{O_1A_1}]_{CT}}{\| \vec{\kappa}_1 \times \vec{O_1A_1} \|}$$

with $[\vec{\kappa}_1 \times \vec{O_1A_1}]_{CT} = \begin{bmatrix} Y_{\kappa_1}(x_{a1} - z_{o1}) - Z_{\kappa_1}(y_{a1} - y_{o1}) \\ Z_{\kappa_1}(x_{a1} - x_{o1}) - X_{\kappa_1}(z_{a1} - z_{o1}) \\ X_{\kappa_1}(y_{a1} - y_{o1}) - Y_{\kappa_1}(x_{a1} - x_{o1}) \end{bmatrix}_{CT}$

and

$$\| \vec{\kappa}_1 \times \vec{O_1A_1} \| = \sqrt{(Y_{\kappa_1}(x_{a1} - z_{o1}) - Z_{\kappa_1}(y_{a1} - y_{o1}))^2 + (Z_{\kappa_1}(x_{a1} - x_{o1}) - X_{\kappa_1}(z_{a1} - z_{o1}))^2 + (X_{\kappa_1}(y_{a1} - y_{o1}) - Y_{\kappa_1}(x_{a1} - x_{o1}))^2}$$

In other words $X_{\kappa_1}, Y_{\kappa_1}, Z_{\kappa_1}$ can be calculated from OsiriX $A_1$, $B_1$ and $O_1$ coordinates.

$\vec{\kappa}_1$ is the normal vector of the dorsal plane (i.e. ventrodorsal axis is perpendicular to the dorsal plane). The cross product $\vec{k}_1 \times \vec{\kappa}_1$ can therefore be used to determine $[\vec{f}_1]_{CT}$.

$$[\vec{f}_1]_{CT} = \left[ \vec{k}_1 \times \vec{\kappa}_1 \right]_{CT} = \frac{[\vec{k}_1 \times \vec{\kappa}_1]_{CT}}{\| \vec{k}_1 \times \vec{\kappa}_1 \|}$$

with $[\vec{k}_1 \times \vec{\kappa}_1]_{CT} = \begin{bmatrix} Y_{\kappa_1} Z_{\kappa_1} - Z_{\kappa_1} Y_{\kappa_1} \\ Z_{\kappa_1} X_{\kappa_1} - X_{\kappa_1} Z_{\kappa_1} \\ X_{\kappa_1} Y_{\kappa_1} - Y_{\kappa_1} X_{\kappa_1} \end{bmatrix}_{CT}$

and $\| \vec{k}_1 \times \vec{\kappa}_1 \| = \sqrt{(Y_{\kappa_1} Z_{\kappa_1} - Z_{\kappa_1} Y_{\kappa_1})^2 + (Z_{\kappa_1} X_{\kappa_1} - X_{\kappa_1} Z_{\kappa_1})^2 + (X_{\kappa_1} Y_{\kappa_1} - Y_{\kappa_1} X_{\kappa_1})^2}$

In other words $X_{\vec{f}_1}, Y_{\vec{f}_1}$ and $Z_{\vec{f}_1}$ can be calculated from $[\vec{f}_1]_{CT}$ and $[\vec{k}_1]_{CT}$.

This complete STEP 1 of the method which was to determine $[\vec{f}_1]_{CT}$, $[\vec{f}_1]_{CT}$ and $[\vec{k}_1]_{CT}$. 

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STEP 2 – Determination of the transformation matrices between the CT coordinate system and anatomical coordinate systems

The purpose of applying a change of coordinate system is to be able to define a vector with respect to a different basis. In our study the vectors of interests $\overrightarrow{IE}$ are representing implant positions defined by an insertion point (I) and an exit point (E), within OsiriX™.

\[
[I\overrightarrow{E}]_{CT} = \begin{bmatrix} X_{\overrightarrow{IE}} \\ Y_{\overrightarrow{IE}} \\ Z_{\overrightarrow{IE}} \end{bmatrix}_{CT} ; [\overrightarrow{IE}]_{C1} = \begin{bmatrix} X'_{\overrightarrow{IE}} \\ Y'_{\overrightarrow{IE}} \\ Z'_{\overrightarrow{IE}} \end{bmatrix}_{C1} ; [\overrightarrow{IE}]_{C2} = \begin{bmatrix} X''_{\overrightarrow{IE}} \\ Y''_{\overrightarrow{IE}} \\ Z''_{\overrightarrow{IE}} \end{bmatrix}_{C2}
\]

The direction cosine $[DC]$ and direction cosine inverse $[DC]^{-1}$ of a particular anatomical coordinate system are defined by the following equations:

\[
[I\overrightarrow{E}]_{CT} = [DC_{C1}] \cdot X_{\overrightarrow{IE}} ; [\overrightarrow{IE}]_{C1} = [DC_{C1}]^{-1} \cdot X_{\overrightarrow{IE}} ; [\overrightarrow{IE}]_{C2} = [DC_{C2}]^{-1} \cdot X_{\overrightarrow{IE}}
\]

By definition, $[DC_{C1}]^{-1}$ = $\begin{bmatrix} i \cdot \hat{i} & j \cdot \hat{j} & k \cdot \hat{k} \\ i \cdot \hat{i} & j \cdot \hat{j} & k \cdot \hat{k} \\ i \cdot \hat{i} & j \cdot \hat{j} & k \cdot \hat{k} \end{bmatrix}$ and $[DC_{C1}] = [DC_{C1}]^{-1}$

Knowing that $[i]_{CT} = \begin{bmatrix} 1 \\ 0 \\ 0 \end{bmatrix}_{CT}$ ; $[j]_{CT} = \begin{bmatrix} 0 \\ 1 \\ 0 \end{bmatrix}_{CT}$ ; $[k]_{CT} = \begin{bmatrix} 0 \\ 0 \\ 1 \end{bmatrix}_{CT}$

And $\vec{a} \cdot \vec{b} = x_a \cdot x_b + y_a \cdot y_b + z_a \cdot z_b = \cos \theta$ ; $\theta$ being the angle between $\vec{a}$ and $\vec{b}$

This implies that $[DC_{C1}]^{-1}$ = $\begin{bmatrix} X_{\hat{i}} & Y_{\hat{i}} & Z_{\hat{i}} \\ X_{\hat{j}} & Y_{\hat{j}} & Z_{\hat{j}} \\ X_{\hat{k}} & Y_{\hat{k}} & Z_{\hat{k}} \end{bmatrix}$ ; $[DC_{C1}] = [DC_{C1}]^{-1}$

Therefore we can determine $[DC_{C1}]^{-1}$ and $[DC_{C1}]$ by knowing $[i]_{CT}$, $[j]_{CT}$ and $[k]_{CT}$, which completes STEP 2.
STEP 3 – Determination implant vectors coordinates with respect to the anatomical basis $\mathbf{[IE]}_c^1$ or $\mathbf{[IE]}_c^2$

$$
\begin{align*}
[IE]_c^1 &= \begin{bmatrix} X'_{IE} \\ Y'_{IE} \\ Z'_{IE} \end{bmatrix} = [DC_{c1}]^{-1} \begin{bmatrix} X_{IE} \\ Y_{IE} \\ Z_{IE,CT} \end{bmatrix} = \begin{bmatrix} X_{C1} \\ Y_{C1} \\ Z_{C1} \end{bmatrix} = \begin{bmatrix} x_E - x_i \\ y_E - y_i \\ z_E - z_i \end{bmatrix} \\
[IE]_c^2 &= \begin{bmatrix} X'_{IE} \\ Y'_{IE} \\ Z'_{IE} \end{bmatrix} = \begin{bmatrix} X_{C2} \\ Y_{C2} \\ Z_{C2} \end{bmatrix} = \begin{bmatrix} x_E - x_i \\ y_E - y_i \\ z_E - z_i \end{bmatrix} \\
\end{align*}
$$

Therefore we can determine $\mathbf{[IE]}_c^1$ by knowing $[C_{c1}]_{CT}$, $[C_{c2}]_{CT}$, $[k_{c1}]_{CT}$ and $[IE]_{CT}$ which completes STEP 3.

STEP 4 – Determination of implants projected angles with respect to the anatomical planes

Projected angles are directly calculated from $\mathbf{[IE]}_c^1$ and $\mathbf{[IE]}_c^2$ using the following formulas:

$$
\begin{align*}
\theta_{Sagittal} (f_i) &= \tan^{-1}\left(\frac{Y'_{IE}}{X'_{IE}}\right) ;
\theta_{Sagittal} (\tilde{f}_i) &= \tan^{-1}\left(\frac{Y'_{IE}}{X'_{IE}}\right) \\
\theta_{Transverse} (f_i) &= \tan^{-1}\left(\frac{Z'_{IE}}{Y'_{IE}}\right) ;
\theta_{Transverse} (k_i) &= \tan^{-1}\left(\frac{Y'_{IE}}{Z'_{IE}}\right) \\
\theta_{Dorsal} (f_i) &= \tan^{-1}\left(\frac{Z'_{IE}}{X'_{IE}}\right) ;
\theta_{Dorsal} (\tilde{k}_i) &= \tan^{-1}\left(\frac{Y'_{IE}}{Z'_{IE}}\right)
\end{align*}
$$
APPENDIX III - DETAILED METHOD OF OSICS CT ANALYSIS: CALCULATION SHEET USED TO GENERATE 3D DATA BASED ON CT ROI POINTS COORDINATES

Fig A.III.1  Example of calculation sheet used for semi-automated calculation of 3D data (case 6).
The dogs of each group were assigned 2 series of random numbers (see Table A4).

**Step 1 - Sample selection:** Using the 1st generated list, the 4 smallest numbers of each group were selected for analysis. However 2 cases were excluded from the AAI group (leaving only 3 cases in this group), therefore 5 cases were selected from the N-Toy group to maintain the sample size of 12 cases.

**Step 2 – Ordering for CT reading:** The list of 12 cases was ordered by incremental values of random numbers (using the 1st series). The obtained list provided the first CT reading list. Then the 12 cases were reordered by incremental values of random numbers using the 2nd series. This provided the random list for the second CT reading.

**Step 3 – Implant selection:** To determine which implant was to be evaluated for each case, 1 permutation of 0 to 9 numbers was generated for each group (see 0). Implant sites were also numbered 0 to 9 with even numbers representing the right side and odd numbers the left. In each permutation list only the first occurrence of an implant was kept (as right and left sides were considered the same implant site). For instance, if the implant number 2 occurred before number 3 only 2 was selected. This resulted in an ordered random list of 5 different implants sites for each group. The implant numbers were then assigned to cases in the order of their occurrence using the case list ordered based on the 1st set of random numbers.
Table A.IV.1 Random numbers generated to allow randomization of the sample used for validation of the mathematical model.

<table>
<thead>
<tr>
<th>Groups</th>
<th>1st random number lists</th>
<th>2nd random number lists</th>
<th>Implant permutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-Beagles</td>
<td>1 -th Random Z = 0.799986756505282</td>
<td>2 -th Random Z = 2.616565167530704</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2 -th Random Z = 0.163618000091536</td>
<td>2 -th Random Z = 0.651351495682784</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>3 -th Random Z = -0.262456591700723</td>
<td>3 -th Random Z = -0.434679436795688</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>4 -th Random Z = 0.603692151102234</td>
<td>4 -th Random Z = 0.081782965859530</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>5 -th Random Z = -1.086257739704893</td>
<td>5 -th Random Z = -0.042881628679629</td>
<td>5</td>
</tr>
<tr>
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<td>6 -th Random Z = 1.842316836137238</td>
<td>6 -th Random Z = -0.831525087587258</td>
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</tr>
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<td>7 -th Random Z = -0.085930353363851</td>
<td>7 -th Random Z = 0.46954106835320</td>
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<tr>
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<td>8 -th Random Z = -0.391439085681254</td>
<td>8 -th Random Z = -0.19847573782175</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>9 -th Random Z = 1.464871655830500</td>
<td>9 -th Random Z = 0.527183320516184</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>10 -th Random Z = -1.306759620000758</td>
<td>1 -th Random Z = 0.286282316156068</td>
<td>10</td>
</tr>
<tr>
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<td>1 -th Random Z = -0.793512930058507</td>
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<tr>
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<td>2 -th Random Z = 1.443303272929233</td>
<td>2 -th Random Z = 0.071846098703032</td>
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</tr>
<tr>
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This simplified estimation was based on the assumption that all the weight of the head is applied directly to the atlantoaxial joint and that the head’s center of gravity is located at mid-level of its rostrocaudal axis. Considering a dog at rest in a standing position, the ventral bending moment (VBM) applied by the head on the atlantoaxial joint can be calculated using the following formula:

\[ \| \overrightarrow{VBM_{\text{Head}}} \| = m_{\text{Head}} \cdot \| \overrightarrow{g} \| \cdot \frac{L_{\text{Head}}}{2} \quad \text{with} \quad \| \overrightarrow{g} \| = 9.81 \text{ m.s}^{-2} \]

The mass \( m_{\text{Head}} \) and length \( L_{\text{Head}} \) of 10 Beagle cadaver heads was measured in order to estimate the physiological values of ventral bending moments applied by the head in Beagle dogs. The following table summarizes these results:

<table>
<thead>
<tr>
<th>Dog</th>
<th>Mass of the head (Kg)</th>
<th>Length of the head (m)</th>
<th>Estimated physiological VBM (N.m)</th>
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<td>0.74</td>
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<td>0.17</td>
<td>1.00</td>
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<tr>
<td>3</td>
<td>0.80</td>
<td>0.18</td>
<td>0.71</td>
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<tr>
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<td>0.80</td>
<td>0.18</td>
<td>0.71</td>
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<td>0.34</td>
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<tr>
<td>Mean</td>
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<tr>
<td>SD</td>
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<td>0.01</td>
<td>0.20</td>
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</table>

Table A.V.1 Values of mass and length measurements from 10 Beagle cadaver heads used to estimate physiological values of ventral bending moments (VBM) applied by the head on the AA joint.

SD: Standard deviation.