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Post Mortem Vitreous Electrolyte Analysis as an Adjunct in the Diagnosis of Salt Water Drowning

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Abstract

Drowning is a potential cause of death in a deceased individual immersed in water. The diagnosis of drowning can be challenging because the post mortem findings are variable, transient, and non-specific and, thus, non-diagnostic. Traditionally, contextual investigation followed by a full post mortem examination has been required for all suspected drowning deaths. However, in cases of strong contextual evidence and the family’s objection to a full post mortem examination, the availability of a non-invasive adjunct test to aid the diagnosis of drowning would be ideal. Although it was previously studied and subsequently abandoned in the 1970s and 1980s, post mortem vitreous electrolyte analysis (a non-invasive test) has recently been re-examined and showed promising results. This thesis examined and established post mortem vitreous electrolyte analysis as a useful adjunct in the diagnosis of salt water drowning.

Three sections are presented that examine different aspects in the use of post mortem vitreous electrolytes as an adjunct in the diagnosis of salt water drowning. Each section contains published original studies and illustrative case(s). The first section established that the post mortem vitreous sodium and chloride (PMVSC) level increases, first from salt water drowning and subsequently from immersion. It demonstrated that PMVSC is a useful adjunct in the diagnosis of salt water drowning when the immersion time is less than 1 hour. The second section established that the post mortem vitreous magnesium (PMVM) level increases from salt water immersion, but not from drowning, which is different from the changes in the PMVSC. Because of this difference, PMVM can be used to assess the effect of immersion when the immersion time is greater than 1 hour or is unknown to aid in the interpretation of PMVSC levels. The third section demonstrated that combining PMVSC and lung weight (in the form of lung-body ratio, LB) provides greater diagnostic certainty for salt water drowning death compared to using either PMVSC or LB alone.

The results have practical implications for the approach to salt water drowning deaths. The traditional approach of performing a full post mortem examination in all cases of suspected drowning deaths is challenged and may not be necessary. The use of post mortem vitreous electrolytes provides a less invasive approach that can be used in selected cases. Furthermore, the methodology and analytical techniques presented in this thesis can be extended to fresh water drowning deaths and non-drowning deaths.
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Chapter 1. Introduction

1.1. Drowning

1.1.1. Historical background of drowning

To determine the cause of death of a deceased individual recovered from water is a major challenge in forensic pathology. The oldest existing textbook in forensic medicine, “Hsi Yuan Lu”, or “Instruction to Coroners”, was written in China in 1247 AD, and it highlights the difficulties of determining the cause of death when a deceased individual is recovered from water [1]. It noted the need to consider the possibility of death from trauma or natural disease before or upon entering the water, and various diagnostic tools purporting to distinguish drowning from other causes were discussed. In contemporary forensic textbooks, the same questions are highlighted [2, 3]. A deceased individual located in water may have died from natural disease before entering or while in the water; from injuries sustained before, upon entering or while in water; from the effects of immersion other than drowning; or from drowning [3]. The diagnosis of drowning is made when there is sufficient contextual evidence that correlates with post mortem findings [4, 5]. At a practical level, it may be difficult or impossible to provide a definitive cause of death in a deceased individual recovered from water, especially when the event was unwitnessed and/or there was a delay in body recovery with associated decomposition [3]. Furthermore, drowning is not only a diagnosis of exclusion; it can be the final outcome of a number of initiating events, such as natural disease, trauma, and alcohol or drug toxicity [2].

The definition and terminology of drowning has evolved over time. Drowning was previously defined as death from asphyxia within 24 hours of immersion in water [2]. A death after 24 hours was considered “near” drowning [2]. Other modifiers, such as “secondary”, “silent”, “wet-dry”, and “active-passive”, have been used in the past but are now considered ambiguous and outdated misnomers [6-8]. Drowning is currently defined by the World Health Organization as the process of experiencing respiratory impairment from submersion/immersion in a liquid [6]. This definition was deemed simple, inclusive, and specific [6]. The terms “immersion” and “drowning” are not interchangeable. Immersion or immersion death is defined merely as a deceased individual found in water; these terms do not offer any information regarding the cause of death. Conversely, a deceased individual
may not necessarily be found immersed in water after being drowned, for example, if he or she was left behind by a receding tide [9].

1.1.2. Epidemiology of drowning

Drowning is a major worldwide public health concern that claimed approximately 360,000 lives in 2015 [10]. This figure underestimates as much as 50% of drowning deaths as it excludes drownings due to natural disasters, transport-related accidents and intentional drowning deaths from suicide and homicide. Most drowning deaths are accidental and occur in swimming pools, lakes, rivers or seas. Suicide drowning is estimated to account for less than 9% of all drowning deaths, and homicidal drowning accounts for less than 0.5%. Drowning is the third leading cause of unintentional death and accounts for 7% of all injury-related deaths. Although deaths from drowning are more common in low- and middle-income countries, high-income countries are not spared. In Australia, drowning is the leading cause of death among children aged between 1-3 years, and in the United States of America, it is the second leading cause of unintentional injury in children aged 1-14 years. Groups associated with drowning deaths include people with low socioeconomic status, males, unsupervised infants, alcohol users, individuals with medical conditions and tourists who are unfamiliar with local waterways.

In New Zealand, the number of drowning deaths has steadily declined over the last few decades [11]. In 2016, drowning claimed 107 lives, was responsible for 207 hospitalizations and was the fourth leading cause of accidental death. It was estimated that 78 of those drowning deaths were preventable. European males between the ages of 45 and 54 years old had a higher risk of death. The two most common locations for drowning in New Zealand were rivers and beaches, which accounted for more than half of the fatalities, and swimming was the activity most commonly associated with drowning.

1.1.3. Pathophysiology of drowning

1.1.3.1 Historical understanding

The understanding of the drowning process has changed in recent decades. Initial studies performed on dogs during the 1940s and 1950s by Swann shaped the modern understanding of drowning [12-15]. Swann observed electrolyte changes in the blood within minutes during drowning and hypothesized that the differences in osmolality between the immersion medium
and the blood was the driving factor that resulted in death. He noted that in fresh water drowning, the plasma sodium and chloride levels were decreased from haemodilution, and potassium was increased as a result of haemolysis. He suggested that the electrolyte alterations led to ventricular arrhythmia and subsequent death. Conversely, in salt water drowning, sodium and chloride levels were increased from haemoconcentration, with no evidence of haemolysis. He concluded that the electrolyte alteration caused plasma fluid to be drawn out of the lungs, resulting in pulmonary oedema, asphyxia and subsequent death.

1.1.3.2 Current understanding

In the 1960s-1980s, further animal and observational studies were carried out, and Modell was able to demonstrate that death from drowning in humans was not a consequence of electrolyte changes [16-19]. Instead, he showed that victims of “near drowning” deaths rarely aspirate enough water to cause life-threatening electrolyte alteration [16]. These observations changed the understanding of drowning deaths, and the contemporary belief is that death results from the combined effects of hypoxia, hypercapnia and metabolic acidosis secondary to respiratory impairment and asphyxia [20-25].

The events leading to death by drowning are considered to be on a continuum that commences when the airways first sink below the water level [26, 27]. The entire head or body does not need to be immersed, and drowning can occur in quite shallow water or in any liquid. In the early stages of immersion, voluntary breath-holding and laryngospasm can prevent water from being inhaled, although some water may be swallowed [26]. Prolonged breath-holding results in hypoxaemia, hypercapnia and metabolic acidosis [17]. A critical level of hypoxaemia and hypercapnia will be reached, typically at about the three-minute mark, and involuntary inspiration will occur [17]. The inspiration of water prevents effective gaseous exchange, which worsens the hypoxaemia, hypercapnia and acidosis. Typically, this intensifies respiratory effort and causes more water to be inspired into the lungs. Laryngospasms and bronchospasms may occur at this point as the airways may be stimulated by the entry of water and thus act to limit the further ingress of water into the respiratory system. This phenomenon is described as “dry drowning”. Ultimately, this response will be reversed, and more water will be inspired into the lungs. Additional injury to the lungs may result from vomiting and the subsequent aspiration of water swallowed during the earlier stages of drowning.
The respiratory, cardiovascular and central nervous systems are the main systems affected by drowning [23]. In the lungs, the inhaled fluid can disrupt gaseous exchange across the alveolar-capillary membrane and cause a decrease in lung compliance [17, 28]. Decreased lung compliance results when the inhaled water affects normal surfactant function, leading to ventilation-perfusion mismatch and the shunting of blood [28]. This leads to hypoxaemia, cardiac depression, and pulmonary vasoconstriction with altered pulmonary permeability, resulting in pulmonary oedema and asphyxiation [17, 28].

The main impact on the heart is from asphyxia, which leads to hypoxia and acid-base imbalance [23]. The change in circulatory volume that results from drowning (an increase in cases of fresh water drowning, and a decrease in cases of salt water drowning) is transient and insignificant compared to the associated asphyxia [29, 30]. Hypoxia and the associated acid-base alteration, together with catecholamine release, adversely impact the heart rate and myocyte contractility [23]. This places the heart at risk of fatal arrhythmia, such as ventricular fibrillation [23]. However, immediate death from arrhythmia due to drowning is uncommon in humans [23]. Irreversible hypoxic brain damage typically occurs within 3-5 minutes when an individual is asphyxiated, as occurs in drowning, and the variability depends on body temperature and the effectiveness of any resuscitation attempts [31, 32].

Other potentially lethal late complications following drowning include metabolic derangement, multi-organ failure, rhabdomyolysis and coagulopathy. The nature of the inhaled fluid can also cause direct infective or chemical lung damage, which may occur at a later stage if the individual survives the initial asphyxia episode due to early life-saving measures.

1.1.4. Diagnosis of drowning

The modern approach to the diagnosis of drowning is to view it as an “equation” in which the end result is drowning and human and environment factors are variables [5]. With this approach, multiple questions must be addressed: Why and how did the deceased individual enter the water? Was the deceased individual dead or alive when he or she entered the water? Why was the individual unable to survive while in the water? Did the deceased individual drown or die from another cause? Traditionally, a diagnosis of drowning is made after a detailed review of all the contextual information, post mortem findings, and all ancillary investigations [4, 5].
1.1.4.1 Contextual evidence

The investigation of a deceased individual recovered from water starts with the ante mortem information gathering and scene examination. Basic ante mortem information includes the identification of the deceased individual. This information can enable access to the individual’s medical, psychiatric and substance use histories. Witness statements and accounts of the deceased individual’s physical and psychological states (if available) may provide information on why and how the deceased individual entered and was unable to remove him or herself from the water.

The scene examination is typically performed by police and specialized scene investigators; occasionally, pathologists might attend. The scene examination is designed to gather as much evidence as possible regarding the deceased individual and the surroundings. A wealth of information can be obtained from the scene examination. With respect to the deceased individual, information such as the condition and location of the body, the state of his or her clothing, signs of trauma, and the presence of life saving equipment can be useful to guide further investigations. The body of water may be natural (such as a river, lake, or sea) or manmade (such as a bathtub, swimming pool or spa). In river, lake or sea environments, the surrounding land topography, weather, and water temperature and conditions should be recorded and reviewed. When a deceased individual is found in a bathtub or shower, he or she may have evidence on him or herself, such as the presence of soap or shampoo, explaining why he or she was found in that particular location. Spas, pools and surrounding electrical appliances should be checked for electrical faults. Drownings that occur in swimming pools should prompt the investigator to determine whether a lifeguard was on duty, whether video surveillance is available and whether any witnesses were present. Other ancillary reports, such as technical diving reports, medical records, safety reports for equipment and watercraft, coast guard reports and ambulance reports should be requested and reviewed if available. Although such investigations are not always completed prior to the post mortem examination, it is important to review the information at hand as it can guide the pathologist on how the post mortem examination should proceed.

1.1.4.2 Macroscopic and microscopic post mortem findings
Post mortem examination is an important part of the investigation of any deceased individual found in or adjacent to water. One of the main goals of post mortem examination is to determine the cause of death and exclude other pathological processes. In terms of drowning, there is no single diagnostic test. The post mortem examination can only offer “clues” or “findings” as to whether the deceased individual has drowned [4]. Typically, the diagnosis is based on a constellation of post mortem findings, but these findings can be variable, transient, non-specific and not always diagnostic, and some signs are merely indicative of immersion [33]. The post mortem findings and other investigations should support the known features seen in drownings and, if absent, an alternative diagnosis should be considered.

The most common post mortem findings in drowning are observed in the respiratory system, and one such common finding is the “plume of froth” in the mouth and nostrils, which has been recognized for centuries [1]. This froth is white or blood-tinged and is composed of fine air bubbles comprising a mixture of the drowning liquid, pulmonary oedema fluid, bronchial secretions and pulmonary surfactant [23]. During respiratory effort, this froth reaches the upper airways and is extruded out of the nose and mouth. This sign is only observed in approximately one-fifth of drowning deaths, typically when the deceased individual is retrieved from the water within a relatively short time frame [33]. A “bubbling froth” that is morphologically similar to this “plume of froth” is also evident in deaths following cardiogenic pulmonary oedema and epilepsy and in drug-related deaths [33].

The lower respiratory findings in individuals who have drowned include lungs that are waterlogged, voluminous, or overexpanded and are often associated with pleural effusion [3, 23, 33, 34]. These findings are frequently associated with an increase in lung weight, which may exceed 1 kg [3]. The absolute lung weight has limited diagnostic value because there is a large range among individuals who have died from drowning and those who have died from other causes [35-41]. The overextension of the lungs can rupture alveolar capillaries and produce a blotchy, haemorrhagic visceral pleural surface, but this sign, too, has limited diagnostic value and can be interpreted as post mortem lividity. Copious white, pink or red froth and fluid can exude from the large and small airways and lung parenchyma when the lungs are sectioned, but such findings are often observed with other causes of pulmonary oedema and thus have limited diagnostic value. Microscopically, the lungs may show intra-alveolar haemorrhagic oedema in a patchy to confluent pattern and focal acute emphysema with alveolar dilatation and thinning and lacerated septa, described as emphysema aquosum.
However, these microscopic lung findings are variable and, again, have limited diagnostic value [3, 23, 33].

Other features sometimes seen in drowning deaths, such as congested viscera, water in the stomach, haemorrhage in the neck muscles from agonal convulsions, liquid in the sphenoid sinus and haemorrhage in the mastoid air cells and middle ear mucosa, are not specific to drowning and have limited diagnostic value [3, 23, 33].

1.1.4.3 Ancillary tests

Numerous biological and biochemical indices have been evaluated as adjuncts for establishing the diagnosis of drowning but are generally considered to have limited diagnostic value because they are often labour intensive, unreliable and non-specific [3, 23, 33]. Examples include measuring the amount of haemodilution, quantifying the biochemical constituents of the lung alveoli, bodily fluids and solid organs.

Water, suspended particles, microorganisms and dissolved chemical substances in water, as well as corpuscular and cellular elements in the lung alveoli, may enter the circulation, and this phenomenon underlies the rationale for the many proposed ancillary tests for drowning [33]. Equally, large quantities of water can be inhaled and/or swallowed during drowning, causing plasma proteins to enter the lungs and solute to shift into the plasma. An example of this phenomenon was recorded in a survivor of drowning following salt water ingestion during a hurricane [42]. The individual was reported to have a serum sodium level of 175 mmol/L and chloride level of 148 mmol/L. Although these alterations have been described in deceased individuals who have drowned, they lack specificity and sensitivity as a result of post mortem autolysis and putrefaction [3, 23, 43]. In 1921, Gettler described a difference in chloride levels between the left and right chambers of the heart in salt water drowning death [44]. This formed the basis of the so-called Gettler test, but this test was subsequently found to be unreliable and is no longer commonly used [18]. In 1944, Moritz suggested blood magnesium as an alternative to chloride as an index of salt water drowning, but this did not achieve widespread application [45]. Instead, strontium became the most widely accepted index to use as a diagnostic test for drowning victims [38, 46-48]. Azparren demonstrated that blood strontium becomes grossly increased in drowning deaths, particularly those resulting from salt water [38, 46-48]. Unfortunately, increased blood strontium is neither a sensitive nor specific index because strontium is not uniform across water bodies, and
increased levels can be found in mineral water drinkers and individuals with a high seafood diet [33]. Blood strontium levels are not routinely measured in Australia and New Zealand.

In the mid-20th century, a series of studies explored the linkage between drowning and the presence of microalgae in the deceased individual’s visceral organs. The method involved searching the visceral organs (particularly the bone marrow) for the presence of a group of microalgae called diatoms, and the presence would indicate the individual had drowned. This “diatom test” is labourious, lacks specificity, and is prone to contamination, making the results difficult to interpret in many cases [3, 23, 49]. A low abundance of diatoms could provide a false low/negative result, whereas prolonged immersion with contamination could yield a false high/positive result. These specialized tests are not performed in Australia and New Zealand and are not considered standard practice elsewhere.

Other ancillary tests that are purported to be useful adjuncts in the diagnosis of drowning include protein levels, surfactant protein expression, aquaporin tissue expression, and genomic identification of waterborne bacteria in tissue [50-53]. Like the diatom test, these tests have not gained widespread acceptance, and most clinical laboratories do not offer them as part of their standard practices.

1.1.5. Historical use of vitreous humour electrolytes in the diagnosis of salt water drowning

The study of vitreous humour electrolytes in forensic pathology is relatively recent, and as a result, its application in death by drowning is not well studied or documented [54-62]. This section will provide an overview of the vitreous anatomy and physiology and the post mortem biochemical changes that might be relevant in deaths from salt water drowning.

1.1.5.1 Anatomy and physiology of the vitreous

The vitreous is the largest structure within the eye and is almost spherical in shape. It comprises some 80% of the ocular volume and occupies the space between the iris-lens diaphragm and the retina. The outer layer of the vitreous is called the hyaloid and is integrated with the ciliary body anteriorly; posteriorly, it is either adjacent to the retinal internal limiting membrane or separated by aqueous fluid if the vitreous is detached. While the vitreous is described as a gel, aged-related changes can manifest as local changes in its consistency. With age, fluid lacunae can develop in some areas, while collagen fibres become
denser in other locations. This process is called syneresis (separation of the component fractions of the vitreous) and can be followed by synchysis (liquefaction of the vitreous and accumulation of cholesterol crystals). These two processes can precipitate a separation of the vitreous from the retina, a condition called posterior vitreous detachment, and are evident both clinically and macroscopically.

The vitreous gel is composed mainly of water, with collagen, proteoglycans, and hyaluron completing the structural matrix [63, 64]. Inorganic and organic substances are dissolved in the water, and there are regional differences within the vitreous. The levels of these dissolved substances are different from those in plasma, resulting in a gradient between the vitreous and plasma. The gradient is maintained by the blood-ocular barrier, ocular metabolism and diffusion processes in the vitreous [63]. Under normal physiological conditions, diffusion is the main pathway through which small molecules enter and leave the vitreous. Bulk flow is more important under pathological conditions, such as penetrating eye injuries or inflammatory eye diseases. Clinically, vitreous fluorophotometry is used to study the solute interchange between blood and vitreous. This investigation involves administering intravenous fluorescein and measuring the amount of fluorescein that enters the vitreous over time. In diabetic retinopathy, for example, the breakdown of the blood-retina barrier facilitates a faster exchange and thus provides a measure of diabetic eye disease.

The factors that determine the rate of solute change in the vitreous are blood-retina barrier permeability and the vitreous diffusion coefficient. The permeability of small solutes across the blood-retina barrier is similar to that of the blood-brain barrier. As mentioned above, conditions such as diabetic retinopathy can impact vascular integrity, which increases blood-retina barrier permeability. In early diabetic retinopathy, the permeability quotient may be increased, but the overall diffusion coefficient is usually unchanged. A transient time constant, or lag, between a change in the blood and vitreous solute levels is in the magnitude of minutes. The diffusion coefficient for solute movement in the vitreous is age dependent. With increasing age, the vitreous becomes more liquid and less gel-like, allowing solute to move more freely within the vitreous body.

Solutes can also enter the vitreous through the sclera, but not through the cornea. The cornea is a transparent structure that acts as a lens, facilitating the entry of light into the eye; it covers approximately 1/6 of the anterior aspect of the eye. The corneal epithelium is impermeable to ions and acts to prevent fluid movement into the corneal stroma. This thwarts
ionic exchange in the vitreous. The sclera is opaque and composes the remainder of the globe. It is permeable to solutes, and its permeability is inversely proportional to the molecular size and thickness of the sclera [54, 65-68]. This route is commonly used for drug delivery into the eyes.

1.1.5.2 Use of vitreous humour biochemistry at post mortem

The study of post mortem vitreous biochemistry was prompted by the work of Coe in the 1970s [43]. Since then, many others have advanced its use, and the substances analysed, variety of techniques used and levels of specificity and sensitivity have improved accordingly [69, 70]. In addition to the vitreous, a number of other bodily fluids have been subject to the same examinations, including blood, urine, cerebrospinal fluid, pericardial fluid and synovial fluid [70]. A range of conditions that were difficult, if not impossible, to diagnose by post mortem examination alone can be complimented with biochemical analysis [69]. Classic examples include anaphylaxis, deranged glucose metabolism, hypothermia, inflammation and sepsis, which can be supported or diagnosed using post mortem biochemical tests [69, 70].

Historically, blood specimens were investigated for post mortem biochemical testing but were not fruitful due to post mortem changes [43, 69, 70]. Vitreous humour is now preferred over blood for most post mortem biochemical testing and is considered the most useful and proven fluid to analyse during post mortem examinations [69]. The vitreous humour has many advantages over blood as it is well contained and remains relatively inert in the eyes. The vitreous is less prone to contamination, and it decomposes more slowly than blood. The vitreous is relatively easy to access, requiring only a needle and syringe [69]. Vitreous is collected by placing a needle through the sclera 4 mm posterior to the corneal-scleral junction and advancing the needle into the mid-vitreous to aspirate the vitreous fluid into the syringe. The target volume is between 2-3 ml, and the fluid is normally crystal clear. Some early reports advocated the pretreatment of vitreous before biochemical analysis because vitreous can be somewhat viscous, especially in younger eyes. Centrifuging, heat treatment and the addition of hyaluronidase have been used [71, 72]. The vitreous fluid can be analysed using the same modern biochemical analytical platforms used for serum. Previous studies reported biochemical differences between the eyes of a single individual, but this difference was subsequently refuted and was probably a reflection of differences in pre-analytical and analytical methods [72].
Post mortem vitreous constituents may change after death; for example, sodium and chloride levels fall, whereas potassium levels increase [73]. The reason for these changes is that vitreous sodium and chloride diffuse into the surrounding cells of the retina and choroid, whereas potassium moves in the opposite direction. Both processes ultimately reach a steady state. Regarding carbohydrate compounds, glucose levels decrease, but ketones remain constant during the post mortem period. Regarding nitrogen compounds, urea and creatinine remain relatively constant. It is then feasible, after accounting for post mortem changes, to diagnose dehydration, hyperglycaemia, ketosis, renal failure, and electrolyte imbalance and to estimate the time of death by analysing vitreous humour electrolytes [70, 74].

1.1.5.3** Historical use of vitreous humour electrolytes in salt water drowning**

Sodium, chloride and magnesium are the three most common ions in salt water. Salt water contains 350-500 mmol/L of sodium and chloride, whereas the magnesium level varies between 50 and 60 mmol/L [42]. Many other electrolytes also exist in salt water, but these will not be further discussed in this thesis. The normal levels of sodium, chloride and magnesium levels in serum are significantly less than those in salt water: Salt water is invariably three to five times higher in sodium and chloride and approximately 50 times higher in magnesium [54, 55, 75, 76]. This differential exists not only for serum but also for vitreous [54, 55, 75, 76]. Clinically and experimentally, during salt water drowning, a large amount of salt water can be inhaled or ingested, resulting in an increase in serum sodium, chloride and magnesium; however, to date, the impact of salt water drowning on the vitreous constituents has not been well documented [16, 19, 77-80].

In the 1970s and 1980s, the use of vitreous as an adjunct to establish the diagnosis of death by drowning was investigated, but the interest was short lived [54, 55, 75, 77]. These initial studies demonstrated that vitreous sodium, chloride and magnesium levels increased in cases of salt water drowning and concluded that these changes were secondary to the effects of passive immersion rather than drowning. A review of drowning published in 2005 only briefly mentioned and dismissed the use of vitreous electrolyte testing in cases of suspected drowning and discussed a number of post mortem blood tests that have been used over the years [23]. These included measurements of changes in serum electrolytes (sodium, chloride, magnesium, potassium) and the detection of exogenous substances in the blood (aquatic organisms, planktons and electrolytes that are present in large quantities in salt water).
Subsequently, there was a complete paucity of studies on the vitreous until the 2010s [56]. Studies conducted in the 1970s and 1980s on the use of vitreous as an adjunct to establish the diagnosis of death by drowning [54, 55, 75, 77] was when the understanding of the pathophysiology of drowning was clarified [16-19]. Interestingly, all these previous studies on vitreous in the context of drowning examined deceased individuals who had been immersed in water for more than 2 hours [54, 55, 75, 77]. Despite a clear understanding of the pathophysiology of drowning – namely, the knowledge that death can occur within minutes - none of these studies examined drowning deaths with shorter immersion periods. In 2011, a study compared vitreous sodium levels in cases of fresh and salt water drowning and observed that sodium levels were increased in cases of salt water drowning and decreased in cases of fresh water drowning [56]. This finding suggested that increased vitreous sodium levels might be a useful test for differentiating between fresh water and salt water drowning. However, the mechanism for this alteration was unclear, and it was suggested that the vitreous sodium changes were secondary to haemoconcentration, haemodilution, or passive immersion.

1.2. The coronial system

1.2.1. History of the coronial system

The office of the coroner, according to current practice in New Zealand, is in charge of investigating sudden unexpected deaths. It has evolved from an English concept that can be traced back to 1194 during the reign of King Richard I. Initially, King Richard created a Crown official who had broader powers, enabling the Crown to maintain control of the justice system in England and to raise revenue through fines. The title given to this official was “Keeper of the Pleas of the Crown” or in Latin custo placitorum cronae. This post was then known as the “crowner” and subsequently as the “coroner”.

Following a major review of the statute of Westminster in 1275, the office of the coroner was restricted to death investigations only. Coroners retain a relative independence from Parliament or the Crown that persists to this day. Their duties have changed from a functional role, such as attending to the deceased individual before the body is removed for burial, to one that has oversight and is less hands-on.

The importance of the coroners’ role was apparent after the Birth and Death Registration Act in 1836, which formalized the need for written documentation from the presiding coroner or
his registrar before a burial can occur. Furthermore, the coroner was required to inform the Crown of all inquest verdicts. This act also empowered the coroner to order a post mortem examination and required a pathologist to attend any enquiry and provide a cause of death.

A complete review of the Coroner’s Act in New Zealand was conducted in 2006, and more recent amendments were made in 2016 [81]. The contemporary role of the coroner, as defined in the act, requires the coroner not only to determine the cause and events surrounding the death but also to provide commentary on reducing the risk of sudden or unexpected death and to promote justice.

1.2.2. Coronial process

The coronial process is governed by the Coroners Act of 2006. The act outlines which deaths should be reported and what process and type of investigation are necessary. The coronial process can be very complex and can involve many interested parties, including the coroners, police, medical professionals and the deceased individual’s families. New Zealand, as a multicultural country, includes specific paragraphs in the act relating to the cultural and spiritual needs of the deceased individual’s family.

The coroner, once notified of a death by the police, must decide whether a post mortem examination is required. If a post mortem examination is deemed unnecessary, the deceased individual can be released to the family. If a post mortem examination is ordered, the deceased individual can then be released once this is completed, after consultation with the forensic pathologist and police, provided the coroner is satisfied with the preliminary findings. The coroner also decides whether to open an inquest if there is a need to establish further evidence to determine the identity of the deceased individual, the cause and manner of death, the time and location of the death, and the events surrounding the death. Finally, the coroner has a duty to make recommendations and provide commentary aimed at reducing similar deaths and ensuring that the public domain is being well served.

In New Zealand, there is one chief coroner, and 20 legally qualified full-time coroners with their own local jurisdictions spread throughout the country. Although legally trained and governed by the same Coroner’s Act, opinions regarding whether a post mortem examination is necessary for a death may vary among coroners.
1.2.2.1 Process of referral to the forensic pathologist

In New Zealand, any person who finds a deceased individual must report the death to the police. The police will initiate a coronial investigation unless there is a valid death certificate. Deaths arising from unknown consequences and deaths that are self-inflicted, unnatural, violent, or related to a medical procedure in which the death was unexpected must be reported. Deaths that occur when an individual is in official custody or care, on a New Zealand aircraft or ship, as well as death during pregnancy or birth are required to be reported to the police. The police advise the coroner and, on behalf of the coroner, undertake an investigation into the reported death. The police investigation will determine the deceased individual’s identity and gather background information, including the events leading up to the death, and will assess whether the death is suspicious. Part of this process may require the police to make formal contact with family members, friends, medical professionals, and witnesses and to acquire any specialists’ reports. The scene will be examined and processed. Finally, the police will present a formal report to the coroner. The coroner’s office may instigate whether a doctor’s death certificate is forthcoming and contact the family to enquire whether there is an objection to a post mortem examination. The coroner’s decision to order a post mortem examination will be based on the police report, the family’s wishes and usually advice from the pathologist. If a post mortem examination is ordered, it is expected that the pathologist will provide a preliminary report to the coroner and will present a final report once any ancillary tests are completed and reviewed.

1.2.2.2 Family objections to a post mortem examination

The Coroners Act emphasizes the recognition of the spiritual and cultural needs of different ethnic groups and the family members of the deceased individual when a post mortem examination is being considered. Unless there are criminal aspects to the death or other legal considerations, any family member can object to a post mortem examination. Equally, the coroner must advise family members of any significant matters related to the legal processes associated with the death. If any member of the family wishes to make an objection, it must be presented as soon as possible, along with a reason for the objection.

If there is no objection from the deceased individual’s family, the coroner can order a post mortem examination if one is deemed necessary. The coroner can overrule any objection from the family if there is a perceived need. Typically, when there is an objection, it is followed by a discussion between the coroner’s office and the deceased individual’s family,
and on most occasions, the family’s objection is withdrawn. The family commonly withdraws their initial objection due to the provision of further information, a change in attitude towards a post mortem examination, or agreement to a less invasive post mortem examination. The objection can be upheld, and the coroner may decide to dispense the death without a post mortem examination. In such instances, the family is advised of the implications of that decision. If the objection is upheld and the coroner still believes that a post mortem examination is indicated, the objection is lodged with the High Court. The High Court has the ultimate ability to either uphold the objection or order the coroner to perform the post mortem examination. To date, there is no case in which an objection has been lodged with the High Court.

1.2.2.3 Decision regarding post mortem examination

The coroner directs a pathologist to perform a post mortem examination, thus enabling him/her to decide whether to open an inquiry. The coroners must consider many facts surrounding the death before making this decision. From the information gathered by police, family members and medical professions, the coroner must determine whether the post mortem examination could disclose any further useful information that is not apparent. Any death that is notifiable under the Health Act 1956 (unnatural death, violent death, suspicious death, and death of public concern) is one for which the coroner may lean towards requesting a post mortem examination. The decision to perform a post mortem examination must be balanced by minimizing the distress imposed on the family. Although they are governed by the Coroners Act, coroners must decide whether a post mortem examination is necessary for each death on its own merits. After the coroner decides that a post mortem examination is necessary, he/she needs to decide what type of post mortem examination is needed, which is again done in close consultation with the police, the family and a pathologist.

1.2.2.4 Types of post mortem examinations

The terms “post mortem examination” and “autopsy” are interchangeable. The word “autopsy” literally means “see for oneself”; an autopsy is a form of medical examination performed by qualified pathologists. Traditionally, the post mortem examination included an external examination, followed by the dissection of the body, including the cranial, thoracic and abdominal cavities [3]. More recently, the use of radiological imaging, biochemical analysis and genetic testing has been incorporated when deemed appropriate. These
investigations also enable the pathologist to focus on regions of the body relevant to the likely cause of death. Sometimes a stepwise approach is taken in the dissection process until the cause of death is ascertained.

The coronial system in New Zealand allows the pathologist to perform varying degrees of post mortem examination depending on the circumstances. Four grades of examination are described:

1. Preliminary inspection, which includes an external examination with radiology and does not allow any samples to be collected or the skin surface to be breeched.

2. Lesser post mortem examination, which includes the preliminary inspection plus the sampling of bodily fluids (such as vitreous humour, blood, cerebrospinal fluid, urine) for ancillary testing via needle aspiration and swabbing the deceased individual’s body surface for trace evidence.

3. Limited post mortem examination, which includes a lesser post mortem examination plus the dissection of a specified body region.

4. Full post mortem examination, which includes external and internal examination with dissection of all the required internal organs and sampling of all the required bodily fluids, tissues and trace evidence.

1.2.3. Challenges in deciding whether to perform a post mortem examination

The decision to perform a post mortem examination and what it might entail can be challenging. If there is a possibility that criminal charges will be made, the police might press for a full post mortem examination. In contrast, when there is no suspicion of foul play or criminal changes, the police might be indifferent to the notion of a post mortem examination. The family of the deceased may object to any form of examination on cultural and spiritual grounds, disregarding any medical or legal reasons. On other occasions, a family may wish to have a post mortem examination, perhaps because of curiosity or for other reasons, despite an absence of medical or legal indications. The forensic pathologist may view the post mortem examination as the last chance to perform any medical investigation and may suggest a full post mortem examination for every death. However, in some cases in which there is a clear medical cause of death, but without a death certificate, the pathologist may decide that a post mortem examination is not warranted. For every death reported to the coroner’s office, the
The coroner must determine whether a post mortem examination is required or indeed necessary. The coroner is charged by the current act to assess expert advice and reports from the police and members of the deceased’s family.

The decision to proceed with a post mortem examination and the extent of that examination can be sources of conflict. The forensic pathologist’s recommendation may be influenced by his or her personal perspective, work load and past experiences. Some pathologists recommend a full post mortem examination for every death referred to the coroner, while at the other extreme, a minimalist approach might be the standard. The lack of consistency and advice that seems at variance with the circumstances, particularly when strong contextual evidence suggests a cause of death, can engender conflict between the coroner and pathologist. There is also a failure of the police, coroners, family members and even medical professionals to understand what can be achieved with a post mortem examination.

Similar to any medical investigation, the post mortem examination has strengths and weaknesses. It can be useful for identifying positive findings to support a certain diagnosis and negative findings to rule out differential diagnoses. There are many false beliefs from non-medical professionals, including the idea that radiology can completely replace a post mortem examination and that the post mortem examination can clarify all uncertainty surrounding the death and that a cause of death can always be established after a post mortem examination.

It would be ideal for the pathologist to offer an evidence-based recommendation regarding whether a post mortem examination is required and to quantify the potential benefits of an invasive post mortem examination. Such recommendations would enable the coroner to make a more informed decision regarding whether and what type of a post mortem examination is necessary, which would reduce the number of unnecessary invasive post mortem examinations and allow the pathologist to focus on deaths that require more attention, decrease exposure risk and, most importantly, respect the needs of the deceased individual’s family.

1.3. Thesis overview

A deceased individual recovered from water may have died from drowning or from another cause, and accurately determining the cause of death is important for medico-legal and social reasons. The traditional approach in suspected drowning deaths is to perform a full post
mortem examination, with dissection of the cranial, thoracic and abdominal cavities and the analysis of bodily fluid for toxicology, biochemical and genetic testing. This approach is necessary to ensure that the most likely cause of death is determined. There is no accepted single test for drowning, and the diagnosis of drowning is a “diagnosis of exclusion”. Conversely, when there is a reliable witness account reporting that the deceased individual had entered the water, was unable to extricate him or herself and had been immersed in the water, the need to perform a full post mortem examination might be questioned. The coroner would deem there is sufficient evidence to conclude death by drowning, especially when the deceased individual’s family objects to a post mortem examination. Many forensic pathologists might argue for a full post mortem examination because of “historical dictates”, but a compromise with the family and coroner may lead to a lesser post mortem examination. The aim of this thesis is to investigate the utility of performing a lesser post mortem examination to provide an index of certainty and avoid the need for a full post mortem examination.

The above sections of this thesis have detailed the application of post mortem biochemistry and evaluated candidate vitreous electrolytes as indicators for salt water drowning death. The next sections of this thesis demonstrate that post mortem vitreous sodium, chloride and magnesium levels are useful indices in the diagnosis of salt water drowning. The diagnostic certainty was increased when vitreous biochemistry was combined with a measurement of lung weight, although the latter is part of an invasive post mortem examination. These are presented in three separate results sections, each of which contains published original studies and illustrative case(s). Section 1 establishes that post mortem vitreous sodium and chloride levels are increased in salt water drowning. This increase arises initially from the process of drowning and subsequently from immersion, making it a useful adjunct in the diagnosis of salt water drowning when the immersion time is less than 1 hour. Section 2 establishes that the post mortem vitreous magnesium level is a reliable indicator of the effects of salt water immersion. Analysing the level of magnesium in the post mortem vitreous can assist in the interpretation of the sodium and chloride levels in suspected salt water drowning deaths when the immersion time is greater than 1 hour or is unknown. Section 3 evaluates the usefulness of combining lung weight (lung-body weight ratio) with the post mortem sodium and chloride levels of the vitreous as a diagnostic indicator of salt water drownings. The findings determined that this is a useful index that provides greater diagnostic certainty than either parameter in isolation.
The data used in the original studies were derived from three different centres: the Department of Forensic Medicine, Newcastle, Forensic and Analytical Science Service, New South Wales Health Pathology, Forensic Science South Australia; and the Department of Forensic Pathology, LabPLUS, Auckland City Hospital. The illustrative case(s) presented in each section are actual salt water drowning deaths from New Zealand (Auckland and Christchurch). The illustrative cases are presented to show how the findings from the original studies in the corresponding sections have aided in the diagnosis of salt water drowning.

The conclusion drawn from these studies is that non-invasive approaches can be a useful adjunct in the diagnosis of salt water drowning when other evidence is forthcoming. While a full post mortem examination can augment the diagnostic certainty, a lesser degree of evidence might be acceptable in certain cases. This conclusion has practical implications for pathologists, coroners, police and family in deciding the post mortem examination approach to use in cases of suspected salt water drowning death.

Furthermore, the concept of combining an ancillary test, such as vitreous electrolyte levels, with morphological post mortem examination findings to improve diagnostic certainty is novel to forensic pathology. This concept has potential widespread application beyond the implications of salt water drownings; it prompts further studies of parameters that may alter as a consequence of death and linking them to improve certainty regarding the cause of death for the deceased individual’s family and the medico-legal authorities.
Section 1: Establishing vitreous sodium and chloride levels as an adjunct in the diagnosis of salt water drowning

This section contains three background studies that establish post mortem vitreous sodium and chloride levels as a useful adjunct in the diagnosis of salt water drowning (SWD). The first study, published in the American Journal of Forensic Medicine and Pathology (Cala AD, Vilain R, Tse R. Increased post mortem vitreous sodium and chloride levels distinguish saltwater drowning (SWD) deaths from immersion deaths not related to drowning but recovered from saltwater (DNRD). Am J Forensic Med Pathol. 2013 Jun;34(2):133-8), compared vitreous sodium and chloride levels between SWD deaths, non-immersion deaths and deaths not due to drowning in which the deceased individual was recovered from salt water (DNRD). This study, reproduced in this thesis as chapter 2, showed that post mortem vitreous sodium and chloride levels were increased in SWD compared to non-immersion deaths and DNRD. This study recognized that the increase in post mortem vitreous sodium and chloride levels in confirmed salt water drowning cases was distinct from the changes in cases of salt water immersion, indicating that vitreous analysis can be an adjunct in the diagnosis of SWD.

Potential confounding factors in the vitreous analysis of a deceased individual immersed in salt water are the effects of immersion on post mortem vitreous sodium and chloride levels. The second study, published in the American Journal of Forensic Medicine and Pathology (Anne S, Tse R, Oldmeadow C, Attia JR, Cala AD. Immersion of Bovine Eyeballs After 1 Hour in Seawater Does Not Result in Increase of Post mortem Vitreous Humour Sodium and chloride Levels. Am J Forensic Med Pathol. 2016 Jun;37(2):108-11), evaluated the effects of salt water immersion using enucleated bovine eyes. This study, reproduced in this thesis as chapter 3, demonstrated that salt water immersion caused an increase in vitreous sodium and chloride levels, but only after 1 hour of immersion. If it is correct that bovine and human eyes share the same physical properties, then it would be reasonable to state that any increase in vitreous sodium and chloride levels in a deceased individual immersed in salt water for less than 1 hour would result from drowning and not immersion.

The final background study in this section, published in the Forensic Science International (Garland J, Tse R, Oldmeadow C, Attia J, Anne S, Cala AD. Increase of post mortem vitreous humour sodium and chloride levels can be used as a reliable test in cases of suspected salt water drowning when the immersion times are less than 1-hour. Forensic Sci
Int. 2016 Sep;266:338-3), further tested the background methodology and results of the observed changes in human vitreous sodium and chloride levels in cases of salt water drowning where the duration of immersion is documented to be less than 1 hour. This study, reproduced in this thesis as chapter 4, showed a significant increase in post mortem vitreous sodium chloride levels in cases of SWD when the immersion time was less than 1 hour, as predicted from the hypothesis derived from the initial two studies.

The overall finding of these three studies was that post mortem vitreous sodium and chloride levels increase in cases of SWD. The increase is initially due to the effects of drowning; subsequently, after 1 hour, it is caused by immersion. A proposed cut-off was established to enable the pathologist to use post mortem vitreous sodium and chloride levels as an adjunct in the diagnosis of SWD when the immersion time was less than 1 hour.

This section concludes with an illustrative case that was published as a case report in the American Journal of Forensic Medicine and Pathology (Tse R, Garland J, Kesha K, Morrow P, Elstub H, Cala A, Spark A, Stables S, Sage M. Increased Post mortem Vitreous Sodium and chloride Level in a Salt Water Drowning Death During Self-Contained Underwater Breathing Apparatus Diving With Diving Mask in Place: Case Report. Am J Forensic Med Pathol. 2018 Feb 16). This case report, reproduced in this thesis as chapter 5, presents a self-contained underwater breathing apparatus (SCUBA) diver who drowned in salt water. The body still had the face masked correctly fitted when it was retrieved, which eliminated the potential changes in vitreous electrolyte levels as a result of immersion. The vitreous sodium and chloride levels were elevated, and concluded that this increase was solely due to the effects of SWD.
Chapter 2. Increased vitreous sodium and chloride levels in salt water drowning death


The original idea for this initial study came from Cala AD, who also managed the project, collected the data and submitted the manuscript. The data analysis was performed jointly by Vilain R and myself. The manuscript was jointly written and reviewed by Cala AD and myself. All three authors were involved in responding to reviewers.

This initial study demonstrated that post mortem vitreous sodium and chloride levels (PMVSC, the sum of vitreous sodium and chloride levels) increased as a result of SWD rather than immersion. The study protocol was designed to compare the vitreous sodium, chloride and PMVSC levels of deceased individuals who had drowned in salt water (SWD) with those who died in salt water but did not drown (DNRD, death not related to drowning). This study demonstrated that PMVSC increased in salt water drowning and was a superior test compared to sodium or chloride levels alone.

2.1. Materials and methods

A five-year retrospective study conducted between 2007 and 2012 of all salt water-related immersion deaths examined by one author (Cala AD) in two forensic departments in Australia (Department of Forensic Medicine, Newcastle, Forensic & Analytical Science Service, New South Wales Health Pathology and Forensic Science South Australia) was performed. All cases underwent a full post mortem examination with toxicology and biochemical analysis.

2.2. Case and control selection

The cause of death for each of the cases was determined to be SWD or DNRD based on the post mortem examination results alone. Randomly selected non-immersion deaths (controls) during the same period that underwent the same selection criteria, vitreous collection method
and biochemistry studies were identified as control cases. The sodium level, chloride level and PMVSC of the SWD, DNRD and control groups were recorded.

2.2.1. Exclusion criteria

Cases were excluded if there was significant bodily decomposition because of the likely absence of vitreous. Similarly, we also excluded cases in which there was suspected or known activity or disease processes that might have led to pathological alterations of serum sodium and chloride levels prior to death. These included water or salt intoxication, dehydration, or any acute diabetic complications.

2.2.2. Vitreous electrolyte analysis

The post mortem vitreous electrolyte analysis was performed by collecting vitreous samples prior to evisceration. A sample was collected from each eye, placed into a plain tube, spundown and analysed on an Olympus 5400 Auto-analyser using the ion selective electrode method. The sodium and chloride levels (mmol/L) were recorded and added to provide the PMVSC. After vitreous collection, the post mortem examination was performed as per usual.

2.2.3. Statistical methods

JMP 9 (SAS, NC, USA) was used for statistical analysis.

To determine whether parametric statistical analysis was appropriate, the data distribution was assessed using normal quartile plot analysis. The data plots for vitreous sodium, chloride and PMVSC were outside the Lilliefors confidence bounds and were considered to have a non-parametric distribution. The Wilcoxon test was subsequently used as a 2-way comparison for vitreous sodium, chloride and PMVSC in the SWD, DNRD and control groups.

The prediction of SWD versus non-SWD (i.e., the DNRD and control groups) based on sodium, chloride and PMVSC values were made using a single binary classification tree classifier with K-fold cross-validation (recursive partition analysis). K-fold validation works by dividing the original data into K subsets. In turn, each of the K sets is used to validate the model fit for the rest of the data, and the model that produces the best validation statistic is selected as the final model. All classification trees were generated using 5-fold cross-validation. The classifier tree’s discriminatory accuracy was obtained by plotting the receiver
operating characteristic (ROC) curve and calculating the area under the ROC curve (AUC). The AUC value is a common method for comparing the discriminatory abilities of different classifier models. This area is a measure of the probability of correctly classifying two randomly drawn pairs into their respective groups.

### 2.3. Results

A total of 23 salt water-related deaths were identified. There were 15 cases of SWD (M:F: 11:4, mean age 41.6 years) and eight cases of DNIRD (M:F: 7:1, mean age 44.5 years). Fifty (50) randomly selected control cases (coronal deaths from a variety of other non-immersion causes) were identified (M:F: 27:23 mean age 50.6 years). The post mortem interval (from time of death to post mortem examination) for all groups was less than 24 hours in most instances, but in some cases, it was not known with certainty. One case in the DNIRD group was excluded from the study due to incomplete electrolyte data. In the DNIRD group, the time immersed in salt water was between several minutes, to up to approximately eight hours. The DNIRD group comprised of four natural deaths (three deaths from ischemic heart disease and one from chronic airways disease) in which the deceased were witnessed to fall into salt water. Two cases were witnessed traumatic deaths from coming into contact with the hull and propeller of a boat resulting in non-survivable blunt force head injury and multiple injuries respectively. One case was a hanging death from under a bridge in which the ligature subsequently torn resulting in the deceased immersed in salt water. The final case was a self-contained underwater breathing apparatus (SCUBA) diving related death in which the diver ran out of oxygen and died from hypoxia in salt water. All cases in DNIRD group did not show signs of drowning at post mortem examination. The biochemical testing results are shown in table 2.1 and figures 2.1-2.3.

<table>
<thead>
<tr>
<th></th>
<th>Na</th>
<th>S.D.</th>
<th>Cl</th>
<th>S.D.</th>
<th>PMVSC</th>
<th>S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>SWD (n=15)</td>
<td>157.6</td>
<td>10.96</td>
<td>138.8</td>
<td>12.98</td>
<td>296.4</td>
<td>22.66</td>
</tr>
<tr>
<td>DNIRD (n=7)</td>
<td>129.14</td>
<td>16.97</td>
<td>113.43</td>
<td>9.88</td>
<td>242.57</td>
<td>26.27</td>
</tr>
<tr>
<td>control (n=50)</td>
<td>135.52</td>
<td>11.49</td>
<td>115.88</td>
<td>9.61</td>
<td>251.4</td>
<td>18.67</td>
</tr>
</tbody>
</table>

**Table 2.1:** Summary of the sodium, chloride and post mortem vitreous sodium chloride (PMVSC) levels in the salt water drowning (SWD), immersion deaths not related to drowning but recovered from salt water (DNIRD) and control groups.
Figure 2.1: Post mortem vitreous sodium chloride (PMVSC, mmol/L) levels in the salt water drowning (SWD), immersion deaths not related to drowning but recovered from salt water (DNRD) and control groups (* p-value <0.0001, *** p-value <0.0004).

Figure 2.2: Vitreous sodium levels (mmol/L) in the salt water drowning (SWD), immersion deaths not related to drowning but recovered from salt water (DNRD) and control groups (* p-value <0.0001, ** p-value <0.0003).
Figure 2.3: Vitreous chloride levels (mmol/L) in the salt water drowning (SWD), immersion deaths not related to drowning but recovered from salt water (DNRD) and control groups (* p-value <0.0001, *** p-value <0.0004). The mean sodium, chloride and PMVSC levels were significantly higher in the SWD group compared to the DNRD and control groups (p-value <0.001). No significant differences were observed between the DNRD and control groups. Recursive partition analysis with 5-fold validation identified PMVSC as a superior discriminator compared with sodium or chloride alone in the determination of SWD. PMVSC values greater than 284 mmol/L were observed in 86.7% of the SWD cases, and none of the SWD cases had PMVSC values less than 258 mmol/L. An intermediate range of 258-284 mmol/L represents an overlap zone with only a small but significant probability of encountering an SWD case (7.8% probability). These figures correspond to a classification model with an AUC value of 0.95, which indicates an excellent discriminatory model [82]. A summary of the classification model is shown in figure 2.4.

Figure 2.4: Vitreous electrolyte-based classifier tree of predictors of salt water drowning (SWD). The best predictor of SWD was when the PMVSC value exceeded 284 mmol/L. PMVSC values less than 258 mmol/L were not observed in any of the SWD cases.
Intermediate ranges (PMVSC 258-284 mmol/L) were identified in 2 SWD cases (overall ROC AUC value: 0.95).

2.4. Discussion

2.4.1. SWD group

The results showed that vitreous sodium, chloride and PMVSC levels were significantly higher in the SWD group than in the DNRD group and controls provided that the deceased individuals were recovered within 24 hours of death and there was no decomposition. It was hypothesized that the increase in vitreous sodium and chloride levels was mainly caused by the entry of sodium and chloride into the vitreous from the circulation via the inhalation and/or ingestion of salt water. It was also assumed that each person who drowned had normal plasma and vitreous electrolyte levels prior to death.

2.4.2. DNRD and control groups

No statistically significant conclusions could be drawn regarding comparisons of the DNRD and control groups due to the lack of statistical power to show any significant differences. It was estimated that approximately 105 cases would be required to elicit any significant differences between the DNRD and the control group, with a type I error (α) =0.05, type II error (β) = 0.20 and a power of 0.80. However, definite DNRD cases are a rarity, and collecting the required number of cases would probably take more than 50 years. Collaboration with other centres in similar coastal environments could offer an alternative approach. Although more studies are required, our data suggest that the sodium, chloride and PMVSC levels in the DNRD group and control group were within similar ranges. Thus, we assumed that the outer layers of the eye are relatively resistant to the diffusion of sodium and chloride or osmosis across the conjunctiva, cornea or sclera if the open eye is subjected to salt water immersion.

2.4.3. PMVSC

Collecting vitreous sodium and chloride samples is a relatively easy and non-invasive method for obtaining useful diagnostic information. The samples can be easily analysed in a regular hospital biochemical laboratory. The use of vitreous biochemistry in suspected drowning cases is documented and has indicated that vitreous sodium levels can discriminate between SWD and fresh water drowning [56]. Sodium levels were significantly higher in the former
and were within the same range found in this current series [56]. The recursive partition analysis performed in our study revealed that PMVSC was superior to sodium or chloride levels alone for discriminating SWD from DNND and controls.

Our data generated an excellent prediction model using PMVSC (AUC: 0.95), in which a PMVSC above 284 mmol/L was strongly suggestive of SWD, whereas a PMVSC of 258 mmol/L or below was not reliably associated with SWD in deceased individuals recovered from salt water. A PMVSC value between 258 mmol/L and 284 mmol/L was classified as inconclusive or non-informative, and hence, contextual evidence and post mortem examination findings must be more strongly weighted to determine the cause of death.

2.5. Limitations

2.5.1. Case and control selection

The main limitation in this current study was the small number of cases in both the SWD and DNND groups. The same difficulty would exist for any other forensic unit that serves a coastal jurisdiction because of the paucity of immersion deaths. Forensic departments that lack a coastal region would not normally have direct experience with SWDs but may incur fresh water drownings, depending again on their geographic location. In many instances, particularly in tropical or subtropical environments, deceased individuals recovered from salt water are significantly decomposed, and it may be difficult or impossible to obtain sufficient vitreous for analysis.

2.5.2. Variations of electrolyte contents in salt water and vitreous

This study did not examine the electrolyte levels in the salt water from which the deceased individuals were recovered. While there are differences in sodium and chloride levels in the world’s oceans and indeed within the same ocean (due to the influences of rivers, rainfall or glacial activity), as a general rule, the sodium chloride levels in the oceans are three to five times higher than those in either plasma or vitreous. However, the eastern seaboard of Australia, particularly New South Wales, has many coastal “lakes” that have outlets to the sea and are tidal. The presence of brackish areas with lower salinity than sea water cannot be excluded in these water systems. Given the markedly higher sodium and chloride levels in the sea, the overall impact of including deceased individuals immersed in possible brackish areas
is likely to be small. Nonetheless, where there is doubt, analyses of the sodium and chloride levels of the immersion water to confirm its salinity are recommended.

The current study assumed that the baseline plasma and vitreous electrolyte levels in the cases and controls were similar. Although the authors accept this has not been proven, this is a limitation in a study of this type.

2.5.3. Effects of immersion on post mortem vitreous electrolytes

Another potential confounding factor was the effects of immersion on post mortem vitreous electrolytes. Although they are relatively impermeable, the cornea, conjunctivae and sclera are not absolutely impermeable to water and solutes. In post mortem examinations, a degree of decomposition is inevitable, and alterations in vitreous electrolytes due to prolonged salt water immersion are expected. The effect of salt water immersion is explored in the following chapters.

2.6. Conclusion

This study showed that sodium, chloride and PMVSC levels in the SWD group were significantly higher than those in the DNRD and control groups. It was hypothesized that in SWD cases, salt water is inhaled, and the dissolved solutes enter the blood circulation via the lungs and then diffuse quickly into the vitreous. This leads to a significant increase in vitreous sodium, chloride and PMVSC levels. Furthermore, the recursive partition analysis suggested that PMVSC was superior to sodium or chloride levels alone for separating SWDs from DNRDs and controls. PMVSC levels of 284 mmol/L or above in cases where the deceased individual is recovered from salt water strongly suggest a diagnosis of SWD if the context is consistent with drowning. Conversely, PMVSC levels below 258 mmol/L are inconsistent with SWD. Values between 258-284 mmol/L represent cases in which the vitreous PMVSC levels are inconclusive or non-informative, and contextual evidence and post mortem findings are needed to determine the cause of death. The effect of passive immersion in salt water on PMVSC is a major confounding factor that is explored in the following chapters.
Chapter 3. The effects of salt water immersion on post mortem vitreous sodium and chloride levels


I was involved in initiating, managing and executing this study. This included conceiving of the original idea; co-supervising the study; designing the study; applying for ethical approval; securing funding; running the study; writing, reviewing and submitting the manuscript; and subsequently responding to the reviewer’s comments. This study was funded by the Royal College of Pathology Australasia (RCPA).

Chapter 2 showed that post mortem vitreous humour sodium and chloride levels (PMVSC, the sum of the vitreous sodium and chloride levels) may represent a novel method for differentiating deaths from salt water drowning (SWD) from deaths in salt water that were not due to drowning (DNRD, death not related to drowning). It demonstrated that PMVSC in SWD was significantly increased compared to DNRD and non-immersion deaths. It was hypothesized that during salt water drowning, large amounts of salt water are inhaled and/or ingested, causing a rapid shift in electrolytes in the blood/plasma. Vitreous humour, which is more stable in the post mortem period than blood, may reflect this shift in electrolytes in the blood, resulting in a significant increase in PMVSC. Thus, when a deceased individual is recovered from salt water, an increased PMVSC would be consistent with SWD [57].

A confounding process that can cause an increase in PMVSC is electrolyte diffusion and/or osmosis across the outer layers of the eye during direct contact with salt water during immersion [54-56]. A previous study using bovine eyes demonstrated that post mortem vitreous electrolytes can change in instances of prolonged salt water immersion [54]. Although death due to drowning can occur within minutes, there are no known studies that have investigated changes in post mortem vitreous electrolytes as a result of shorter periods of salt water immersion [54-56]. This study investigated the changes in PMVSC, using bovine eyes as a human surrogate, as a result of shorter immersion times in salt water.
3.1. Materials and methods

3.1.1. Case selection

Bovine eyes from freshly sacrificed Hereford cattle (deceased for 2-8 hours) were obtained from a local abattoir. The eyelids were removed, but the extraocular adherent soft tissues were left intact. The eyes were manually randomized into two groups (“wet” and “dry”) and submerged into a plastic tank containing 10 L of salt water collected from a local beach (Merewether Beach on the coast of Newcastle, New South Wales, Australia; sodium: 551 mmol/L; chloride 555 mmol/L). This volume was such that the total volume of bovine eyes comprised less than 5% of the salt water to ensure a steady sodium and chloride level in the salt water. The salt water temperature in the tank was kept steady at 19°C. The eyes allocated to the “wet” group were directly immersed in the salt water (figure 3.1), while the eyes in the “dry” group were placed inside a sealed, impermeable dry plastic bag and then placed in the salt water container (figure 3.2).

Figure 3.1: Arrangement for the “wet” group

Figure 3.2: Arrangement for the “dry” group
3.1.2. Vitreous electrolyte analysis

Vitreous from each group was sampled at 30 minutes, 1 hour, 6 hours and 12 hours by aspirating all the vitreous from each eye (approximately 7-10 ml) with a fresh sterile 16-gauge needle connected to a 20-mL syringe (figure 3.3). The samples were placed in a plain container containing no preservatives and/or additives. The samples were analysed for sodium and chloride by a local accredited biochemistry lab (Pathology North, John Hunter Hospital, New Lambton Heights, New South Wales, Australia). Prior to analysis, the vitreous aliquots were spun down. Sodium and chloride were measured using an ion selective electrode on an Olympus 5400 Auto-analyser.

![Figure 3.3: Method for aspirating vitreous humour from both the “wet” and “dry” groups](image)

The sodium and chloride levels were summed to yield the PMVSC. Using previously published data [54, 57], this study was powered such that a sample of six eyes at each time-point from each group with a standard deviation of 12 mmol/L would provide a power of 80% to detect a 20 mmol/L change in the PMVSC at a 5% statistical significance threshold. Six extra bovine eyes were examined at 0 minutes to obtain a baseline sodium and chloride level.

3.1.3. Statistical methods

SAS v9.4 (SAS Institute, Cary, North Carolina, USA) was used for the analysis.

The data were summarized at each time-point separately for each group using means and standard deviations. Prediction intervals for individual values were also presented for the “dry” group.

The differences between the “wet” and “dry” groups at each post-baseline time-point were determined using permutation tests. These tests are robust to departures in model
assumptions, such as normality and constant variance. Linear regression was used to explore
trends over time in both groups of eyes, with 95% prediction intervals provided for each
time-point.

3.2. Results

Summary statistics for PMVSC at each time-point for the “wet” and “dry” groups and the
between-group differences at each time-point are presented in Table 4.1.

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline (n=6)</th>
<th>30 minutes (n=6)</th>
<th>1 hour (n=6)</th>
<th>6 hours (n=6)</th>
<th>12 hours (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry</td>
<td>Mean (sd)</td>
<td>232 (8)</td>
<td>239 (12)</td>
<td>228 (10)</td>
<td>233 (7)</td>
</tr>
<tr>
<td></td>
<td>Median (min, max)</td>
<td>233 (223, 240)</td>
<td>242 (217, 254)</td>
<td>232 (214, 239)</td>
<td>233 (224, 241)</td>
</tr>
<tr>
<td>Wet</td>
<td>Mean (sd)</td>
<td>235 (9)</td>
<td>238 (5)</td>
<td>304 (37)</td>
<td>401 (37)</td>
</tr>
<tr>
<td></td>
<td>Median (min, max)</td>
<td>237 (221, 245)</td>
<td>241 (230, 242)</td>
<td>301 (260, 367)</td>
<td>393 (370, 470)</td>
</tr>
<tr>
<td>Dry-Wet</td>
<td>4.5, p=0.508</td>
<td>-9.7, p=0.069</td>
<td>-71.0, p=0.006</td>
<td>-174, p=0.003</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.1: Post mortem vitreous sodium chloride (PMVSC) levels summarized at
each time-point as 1) mean (sd) and 2) median (min, max). P-values for differences in
means were computed using permutation tests.

There was weak evidence of a departure in the mean PMVSC between groups at 1 hour
(mean difference = -9.7 mmol/L, p=0.069), but this effect was too small to be clinically
relevant and was not statistically significant at the 5% level. However, the difference was
statistically significant at 6 hours (mean difference = -71.0 mmol/L, p=0.006). In the “dry”
group, there was a slight decline in the mean PMVSC over time (figure 3.4), with the mean
change per hour = -1.68 mmol/L (p=0.1863, R^2=0.06). Shown on the plot in figure 3.4 are the
95% prediction intervals.
Figure 3.4: Post mortem vitreous sodium chloride (PMVSC) level trend over time for the “dry” group, with 95% prediction intervals.

Due to the heterogeneity among the “wet” group in the time trend results, the prediction intervals for these eyes were estimated separately by time ≤ 1 hour and > 1 hour (figures 3.5a and b). A non-significant trend was observed in the first hour (change per hour = 5.7 mmol/L, p=0.196, $R^2=0.1$). The trend increased dramatically after 1 hour, with an hourly increase of 16.3 mmol/L (p=0.001), and explained 67% of the variation in PMVSC.

Figure 3.5a: Post mortem vitreous sodium chloride (PMVSC) trend over time for the “wet” group (time ≤ 1 hour), with 95% prediction intervals.
3.3. Discussion

This study showed that immersion of bovine eyes in salt water for less than 1 hour did not result in a statistically significant change in PMVSC. The changes in PMVSC that occurred after 1 hour, we believe, resulted from a combination of electrolyte diffusion into the vitreous and the movement of water out of the eye by osmosis. A previous study that established the use of bovine eyes as an animal model to assist in the determination of immersion time [54] did not investigate the changes in PMVSC during shorter periods of immersion in salt water, which occur in many drowning deaths.

While immersed, the eyes come in direct contact with salt water, and a high sodium and chloride level gradient is established between the vitreous humour and salt water across the outer layers of the eyes (including the sclera, retina, cornea, aqueous humour and lens). This will cause electrolyte diffusion and/or osmosis to occur across the outer layers of the eye, causing an increase in the PMVSC level as a result of immersion [54, 56]. However, in the present study, immersion did not result in any statistically significant increase in bovine PMVSC for up to 1 hour.

Assuming that human and bovine eyes have similar physical properties, an increased PMVSC in a deceased individual who was immersed in salt water for less than 1 hour should not result from immersion, but from drowning. This warranted a further study of SWD in which the deceased individuals were immersed for less than 1 hour to investigate whether the PMVSC truly increased as a result of drowning in these deaths. This study is presented in chapter 4.
3.4. Limitations

3.4.1. Physical properties of bovine eyes

The main limitation of this study was the assumption that the physical properties of human and bovine eyes are similar. It would not be feasible to harvest the same number of human eyes at a particular time for a similar study. Although the findings for bovine eyes may not be directly translatable to humans, the bovine eye remains a good substitute for this particular type of study. Bovine, porcine and rabbit eyes are commonly used as surrogates for human ocular permeability studies [54, 66-68]. Ocular permeability is inversely proportional to molecular weight and size [65]. The difference in permeability between human and bovine eyes decreases as the molecular weight of the solute decreases [65]. Although there is no single study examining differences in permeability to small elemental molecules between these species, the permeability to sodium, and most likely chloride and water, is considered similar in humans and bovines [65]. Hence, it is reasonable that the enucleated bovine eye is a robust surrogate for humans in salt water immersion studies.

3.4.2. The presence of an eye socket and eyelids

The effects of the presence of an eye socket and eyelids was not investigated in the study. An eye socket and eyelids may provide a more impermeable interface between salt water and the vitreous humour, thus delaying the increase in PMVSC during immersion in salt water.

3.4.3. Physical properties of the salt water used

The salt water used in this study only reflects local sodium and chloride levels. Although salt water around the world is generally three to five times higher in sodium and chloride than blood/plasma and vitreous humour, the extrapolation of the results to salt water with sodium and chloride levels different from those used this study should be performed with caution.

Temperature and water movement were not investigated. The salt water temperature in this study was well controlled at 19°C, which may not reflect water temperatures at different depths. The roles of tides and waves were not modelled in the study. These factors were considered insignificant due to the short duration of immersion.
3.5. Conclusion

There was no statistically significant increase in PMVSC when bovine eyes were immersed in salt water for up to 1 hour. Assuming physical properties similar to those of humans, any increases in PMVSC in a deceased individual immersed in salt water for less than 1 hour should not be due to immersion. This warranted further study of the PMVSC in SWDs in which the deceased individual is immersed for less than 1 hour, as presented in chapter 4.
Chapter 4. Increased post mortem vitreous humour sodium and chloride levels as a useful adjunct in the diagnosis of salt water drowning when the immersion time is less than 1 hour

(Modified from an original article published in Forensic Science International. Garland J, Tse R, Oldmeadow C, Attia J, Anne S, Cala AD. Increased post mortem vitreous humour sodium and chloride levels can be used as a reliable test in cases of suspected salt water drowning when the immersion times are less than 1-hour. Forensic Sci Int. 2016 Sep;266:338-342)

I was involved in initiating, managing and executing this study. This included the conceiving the original idea; co-supervising the study; designing the study; applying for ethical approval; securing funding; running the study; writing, reviewing and submitting the manuscript; and subsequently, responding to the reviewer’s comments. This study was funded by the Royal College of Pathology Australasia (RCPA) and was approved by the Hunter New England Human Research Ethics Committee (Authorization number: AU201602-10).

In chapter 2, the post mortem vitreous humour sodium and chloride levels (PMVSC, sum of vitreous sodium and chloride levels) in salt water drowning deaths (SWD) were compared with those in immersion deaths not related to drowning but recovered from water (DNRD) [57]. These results showed that an increased PMVSC may be a potential adjunct test for the diagnosis of SWD. The proposed mechanism for this increase was that the inhalation and ingestion of salt water in SWD causes a rapid shift in plasma electrolyte levels, a previously documented phenomenon [33, 42] that is reflected in an increase of PMVSC.

A confounding factor that might alter the vitreous electrolytes in salt water immersion cases is changes resulting from diffusion and/or osmosis through the outer layers of the eye. There is good evidence to suggest that prolonged salt water immersion does cause PMVSC to increase [54-56, 75], but the evidence is lacking for shorter immersion timeframes. We investigated the impact of short-term immersion (less than 1 hour) on bovine eyes and found no significant change from the baseline PMVSC [58]. Bovine eye have permeability coefficients similar to those of human eye, which validates this model and enables us to state that there is no increase in PMVSC in human eyes when the immersion in salt water lasts less than 1 hour [58].
This chapter investigated whether there was truly an increase in PMVSC in SWD with immersion times of less than 1 hour (SWD-1) in humans. An increase in PMVSC in SWD-1 would not result from immersion, but from drowning, and would support the hypothesis that during SWD, the inhaled and/or ingested salt water causes electrolyte changes in the blood that are reflected in the increase in PMVSC.

4.1. Materials and methods

A retrospective study comparing the PMVSC in SWD-1 deaths and controls from 2012-2015 (inclusive) was performed at the Department of Forensic Medicine, Newcastle, Forensic & Analytical Science Service, New South Wales Health Pathology.

4.1.1. Case selection: SWD-1 group

All witnessed or clinically confirmed SWD-1 deaths admitted to the department during the study period were selected. Twenty-four SWD-1 cases were identified by selecting all SWD deaths in which there was a clear documentation of an immersion time of less than one hour in the referring police report. The deceased’s age, sex, location of death, events surrounding the death, time between death and the post mortem examination, and vitreous humour sodium and chloride levels were recorded.

4.1.2. Control selection: Non-immersion deaths

In chapter 2, DNRED was used as a control [57], but since then, an experimental study in which sacrificed bovine eyes were immersed in salt water showed that PMVSC was unchanged within the 1-hour time window [58]. Thus, for this study, DNRED cases were no longer needed as controls. Instead, non-immersion deaths in which vitreous humour sodium and chloride were analysed at post mortem examination during the same study period in the same department were sequentially selected as controls. Coronial cases such as sudden unexpected infant deaths, suspicious deaths, homicides, and bodies that were decomposed, incinerated or recovered from water were excluded. The number of controls was four times the number of SWD-1 cases (96 cases), which gave the study 80% power to detect a 6.5-mmol/L difference in PMVSC at the 5% significance threshold (assuming a standard deviation of 10 mmol/L [57]). The deceased’s age, sex, cause of death, time between death and the post mortem examination, and vitreous humour sodium and chloride levels were recorded.
4.1.3. Exclusion criteria

Conditions that could have an impact on the vitreous humour sodium and chloride levels (such as end stage liver disease, hyponatraemia, and diabetic ketoacidosis or any other case of suspected metabolic derangement) were excluded.

4.1.4. Vitreous electrolyte analysis

All vitreous humour samples were analysed for sodium and chloride, after being spun down, by a local accredited biochemistry lab (Pathology North, John Hunter Hospital, New Lambton Heights, New South Wales, Australia) using an ion selective electrode analyser (Abbott chemistry analyser C16000/C8000 or Olympus 5400 auto-analyser). In both the SWD-1 and control groups, the PMVSC was calculated by adding the sodium and chloride levels of the vitreous humour.

4.1.5. Statistical methods

SAS 9.4 (SAS Institute, Cary, North Carolina, USA) was used for analysis.

Continuous variables were described using means and standard deviations. Medians with minima and maxima were presented. Categorical variables were described using counts and percentages for cases and controls. Differences between cases and controls in age, time between death and post mortem examination, vitreous humour sodium, vitreous humour chloride, and PMVSC were assessed using Student’s t-test, and between-group differences in sex were assessed using the Pearson chi-square test.

Logistic regression models were used to assess the predictive ability of the vitreous humour sodium, vitreous humour chloride, and PMVSC measures. A baseline logistic regression model including the variables that differed between cases and control was first created. The incremental improvement in fit (according to the likelihood ratio test) and discriminatory ability (according to the area under the curve in the plotted receiver operating curve) was assessed by separately adding vitreous humour sodium, vitreous humour chloride, and PMVSC to the baseline model. Optimal cut-points chosen to minimize the Euclidean distance to the perfect predictor (a sensitivity of one and a false positive rate of zero) were presented for all measures. Sensitivity, specificity, and positive and likelihood ratios were also presented for the optimum cut-points.
4.2. Results

For the 24 cases of SWD-1 identified during the study period, the average age was 40 years (range: 7-70), and males predominated (M:F: 19:5). The average time between death and the post mortem examination was 2.9 days (range: 1-6 days). The locations of the deaths were local beaches along the east coast of Australia facing the Tasman Sea, a part of the Pacific Ocean. The most common reason for entering the sea was recreational swimming (14 cases), followed by accidentally falling or being washed off rocks into the sea when fishing (4 cases), diving (2 cases of SCUBA diving; 1 case of non-SCUBA diving), and surfing (2 cases).

For the 96 sequential controls, the conditions excluded in the study were diabetic ketoacidosis, end stage liver disease, insulin toxicity, pancreatitis, and hyponatraemia. The average age was 51 years (range: 7-98), and there was a male predominance (M:F: 58:38). The average time between death and the post mortem examination was 3.3 days (range: 1-6 days).

The SWD-1 cases differed significantly from the controls in terms of PMVSC and vitreous sodium and chloride levels (table 4.1).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Level</th>
<th>Case (n=24)</th>
<th>Control (n=96)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>Female</td>
<td>5 (21%)</td>
<td>38 (40%)</td>
<td>0.0866</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>19 (79%)</td>
<td>58 (60%)</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>Mean (sd)</td>
<td>40 (18)</td>
<td>51 (16)</td>
<td>0.0036</td>
</tr>
<tr>
<td></td>
<td>Mean (sd)</td>
<td>2.9 (1.2)</td>
<td>3.3 (1.5)</td>
<td>0.0994</td>
</tr>
<tr>
<td>Days to post mortem</td>
<td>Mean (sd)</td>
<td>146 (9)</td>
<td>129 (10)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Median (min, max)</td>
<td>148 (128, 159)</td>
<td>129 (110, 160)</td>
<td></td>
</tr>
<tr>
<td>Vitreous humour test</td>
<td>Na</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean (sd)</td>
<td>125 (8)</td>
<td>109 (10)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Median (min, max)</td>
<td>125 (108, 143)</td>
<td>109 (89, 140)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean (sd)</td>
<td>271 (16)</td>
<td>238 (18)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Median (min, max)</td>
<td>275 (236, 298)</td>
<td>237 (201, 300)</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.1: Summary of SWD-1 and control group results

Vitreous humour sodium, vitreous humour chloride, and PMVSC were very good discriminators of the outcome (SWD-1), with AUCs of 0.895, 0.905 and 0.908 (95% CIs: [0.83, 0.96], [0.84, 0.97], [0.84, 0.97]) for vitreous humour sodium, vitreous humour chloride and PMVSC, respectively (table 4.2). There was substantial improvement in model fit and
discrimination from a baseline model that included only age, sex and time from drowning to the post mortem examination (likelihood ratio (LR) p-value = <0.0001) (table 4.3, figure 4.1). Of all the measures, PMVSC had comparable sensitivity (0.8) and the greatest specificity (0.9) and positive likelihood ratio (7.6).

<table>
<thead>
<tr>
<th>Measure</th>
<th>Cut-point</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive LR</th>
<th>Area under the curve (lower, upper)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>136</td>
<td>0.83</td>
<td>0.78</td>
<td>3.8</td>
<td>0.90 (0.83, 0.96)</td>
</tr>
<tr>
<td>Chloride</td>
<td>119</td>
<td>0.83</td>
<td>0.83</td>
<td>5</td>
<td>0.90 (0.84, 0.97)</td>
</tr>
<tr>
<td>PMVSC</td>
<td>259</td>
<td>0.79</td>
<td>0.9</td>
<td>7.6</td>
<td>0.91 (0.84, 0.97)</td>
</tr>
</tbody>
</table>

Table 4.2: Optimal cut-points for vitreous humour sodium (Na), vitreous humour chloride (Cl) and PMVSC derived from the unadjusted logistic regression models

<table>
<thead>
<tr>
<th>Model</th>
<th>LR test p-value</th>
<th>Area under the curve (lower, upper)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Age+sex+time between death and the post</td>
<td>Ref</td>
<td>0.72 (0.61, 0.82)</td>
</tr>
<tr>
<td>mortem examination</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2) Vitreous humour Na + model 1</td>
<td>&lt;0.0001</td>
<td>0.91 (0.83, 0.98)</td>
</tr>
<tr>
<td>3) Vitreous humour Cl + model 1</td>
<td>&lt;0.0001</td>
<td>0.93 (0.86, 0.98)</td>
</tr>
<tr>
<td>4) PMVSC + model 1</td>
<td>&lt;0.0001</td>
<td>0.93 (0.86, 0.99)</td>
</tr>
</tbody>
</table>

Table 4.3: Improvement in predictive ability of vitreous Na, vitreous Cl and PMVSC derived from the logistic regression models

Figure 4.1: Receiver operating characteristic (ROC) curves for vitreous humour sodium (Na), vitreous humour chloride (Cl) and post mortem vitreous humour sodium and chloride PMVSC (adjusted for age, sex, and time between death and the post mortem examination (days-P)), overlaid with the curve from comparison models

4.3. Discussion

This study compared 24 cases of witnessed or clinically confirmed SWD-1 with 96 controls (non-immersion deaths) from 2012-2015. It clearly demonstrated a significant PMVSC (and vitreous sodium and chloride) increase in SWD-1 cases compared with controls, indicating that PMVSC was a good diagnostic test for SWD. There was no significant difference in the
interval between the time of death and the post mortem between the groups; consequently, this interval was not a confounding factor. The increase in PMVSC (and in vitreous sodium and chloride) in SWD-1 was due to drowning and not immersion. This finding supports the hypothesis that the inhalation and ingestion of salt water during drowning causes a rapid shift in plasma electrolyte levels, which results in a significant increase in PMVSC. This adds to knowledge regarding the changes in vitreous humour electrolytes in deceased individuals immersed in water. Based on this study and the studies presented in chapters 2 and 3 [57, 58], in SWD, PMVSC appears to initially increase as a result of drowning, then plateau for a period of at least 1 hour and subsequently increase due to immersion.

Based on the data generated in this study, the optimal cut-points with corresponding sensitivity, specificity and likelihood ratio (LR) for vitreous humour sodium, vitreous humour chloride and PMVSC were 136 mmol/L (sensitivity = 0.8; specificity = 0.8; LR = 3.8), 119 mmol/L (sensitivity = 0.83; specificity = 0.83; LR = 5.0) and 259 mmol/L (sensitivity = 0.8; specificity = 0.9; LR = 7.6), respectively.

4.4. Limitations

4.4.1. Bovine eyes as surrogates for human eyes in salt water immersion studies

The present study extended the studies presented in chapters 2 and 3 [57, 58]. Similar to the study presented in chapter 3, one of the assumptions was that immersion in salt water for 1 hour would not increase PMVSC in humans, which was based on data observed from immersing sacrificed bovine eyes in salt water [58].

4.4.2. Variable electrolyte levels in salt water and vitreous

Similar to the studies reported in chapters 2 and 3, the results generated in this study were based on cases retrieved from local salt water from local catchment areas; consequently, the studies have similar limitations. Again, similar to previous chapters, the baseline vitreous sodium and chloride levels prior to death were assumed to be similar between SWD-1 and controls, although this cannot be confirmed.
4.4.3. Study population and control selection

Although the epidemiologic data of the sample (age, sex, time between death and the post mortem examination) made a minimal contribution to the observed increase of PMVSC in SWD-1, extrapolation of our data beyond these parameters should be made cautiously.

Our previous study used DNRD cases as controls to study SWD [57], as it was previously unknown whether PMVSC would increase as a result of SWD. Chapter 3 studied sacrificed bovine eyes immersed in salt water and showed that PMVSC was unaffected by diffusion/osmosis within the 1-hour time window [58]. Therefore, the use of DNRD cases as controls was not required in this study.

DNRD cases with an immersion time of less than 1 hour (DNRD-1) would be the ideal controls for SWD-1; however, this was limited by the number of actual cases. During the study period, nine cases of DNRD were identified; of these, only four cases were DNRD-1. The cause of death in these cases was fatal trauma sustained in salt water (M:F: 2:2; age range: 11-50 years; time between death and the post mortem examination: 2-3 days). The PMVSC values in these cases were 226, 244, 257, and 258 mmol/L, for an average of 246 mmol/L. The PMVSC values were comparable to those of the controls and lower than those of the SWD-1 group, which was consistent with the study results.

4.4.4. The immersion period of less than 1 hour

In a significant number of SWD cases, the immersion time is unknown. Our data are limited to deceased individuals with an immersion time of less than 1 hour. This chosen time period was based on a previous study presented in chapter 3 and the reasonable assumption that bovine eyes are robust surrogates for humans.

4.4.5. Limitations of a clinical test

Similar to any investigation in medicine, PMVSC should be interpreted in conjunction with the clinical history and post mortem findings. PMVSC is only a reliable adjunct test to add greater certainty to the diagnosis of SWD.

4.5. Conclusion

Significant increases in PMVSC levels occur in SWD deaths with immersion times of less than 1 hour. This increase is due to drowning and not immersion. PMVSC is a reliable
adjunct test that can be used in suspected SWD deaths. A PMVSC of 259 mmol/L has a sensitivity, specificity and likelihood ratio of 0.8, 0.9 and 7.6 respectively, making it a good predictor of SWD when the immersion time is less than 1 hour.
Chapter 5. Illustrative case report


The post mortem examination was performed by Dr Sage (consultant forensic pathologist at Christchurch Hospital). My role in this case report was to prepare the manuscript for publication; consult with my co-authors, who were co-investigators; and submit the manuscript.

Previous chapters in this section demonstrated an increase in vitreous sodium and chloride levels (PMVSC, sum of vitreous sodium and chloride levels) observed at the post mortem examination, suggesting that these parameters might be a useful adjunct test in the diagnosis of drowning [57, 59]. The increase in PMVSC was hypothesized to be secondary to the blood electrolyte changes caused by the inhalation or ingestion of salt water during drowning [57, 59]. However, a major confounding factor is passive immersion, which results from sodium, chloride and water diffusing across the sclera and/or cornea [54, 56-59, 75]. This case report describes a SCUBA diver who drowned in a salt water environment and was still wearing a diving mask when his body was recovered. The importance of the retained diving mask eliminates the possibility that the observed increase in PMVSC (294 mmol/L) resulted from immersion and provides evidence for the hypothesis detailed above.

5.1. Case history

A 64-year-old experienced SCUBA diver planned a dive with a diving buddy to retrieve a submerged mooring. Prior to the dive, the diving gear was checked and was found to be in a satisfactory condition. The diver was initially tethered to a rope connected to a float, but this arrangement was discarded shortly into the dive, and the diver re-surfaced after only descending a few metres. The diver then descended again into murky water where the visibility was restricted to less than two metres. The diving buddy observed a stream of bubbles coming from the diver, but these became irregular and subsequently ceased. The diver never re-surfaced. The diving buddy recorded the depth of the dive as approximately 9 metres and the duration of the dive was 15 minutes. The diving buddy contacted the police,
and a search was undertaken; the deceased was found 6 hours later on the ocean floor. The diving mask was still in place when the body was retrieved. The police did not record any damage to the diving mask or salt water in the diving mask. The diving mask was removed upon retrieval of the body.

5.2. Post mortem finding

A post mortem CT scan was performed on the day of death (less than 8 hours after the death) and found no evidence of air embolism. A post mortem examination was conducted less than 24 hours after death.

External examination showed extensive sea scavenger artefacts (sea lice) on the exposed skin of the face, throat and ears. The covered surface was intact, particularly around the eye region, and the imprint of the diving mask was readily apparent (figure 5.1).

![Figure 5.1: Photo depicting the impression of the diving mask around the face region at the post mortem examination. The mask prevented the orbits to coming into contact with salt water and spared the eye region from sea lice infestation.](image)

The internal examination showed non-specific features of drowning, including mildly inflated and wet lungs (left: 669 g, right: 673 g). The heart showed features of hypertensive and ischaemic changes. The left circumflex and left anterior descending coronary arteries showed moderate to severe atherosclerosis. The heart was enlarged at 495 grams, with left ventricular hypertrophy. The abdominal and cranial cavities were unremarkable. A microscopic examination of the visceral organs was non-contributory.

5.3. Ancillary tests

Iliac vein blood, urine, liver and vitreous samples were sent for toxicology. Carbon monoxide saturation was less than 5%, and no alcohol was detected. The toxicological screen was negative. The vitreous humour sample was centrifuged prior to analysis but was not heat
treated. Sodium and chloride levels were measured using an ion selective electrode analyser (Abbott chemistry analyser C16000). The vitreous humour presented a sodium level of 158 mmol/L and a chloride level of 136 mmol/L, yielding a PMVSC of 294 mmol/L.

5.4. Cause of death

The cause of death was deemed to be SWD on the basis of the witness account and the pulmonary changes found at the post mortem examination, together with the results of supportive ancillary tests, including the increased PMVSC of 294 mmol/L. Ischaemic and hypertensive heart disease may have precipitated or contributed to the death in this case.

5.5. Discussion

This case report documented increased PMVSC in a man who drowned during SCUBA diving. The deceased’s diving mask remained in place, preventing salt water from coming into contact with the eyes and the ocular adnexa and thus eliminating the effects of immersion on the PMVSC. The PMVSC was 294 mmol/L, well above the value required to diagnose SWD (259 mmol/L) [59].

The studies presented in the previous chapters examined PMVSC in human SWD cases [59, 83-85]. It is known that large amounts of salt water are inhaled and/or ingested within minutes during drowning, and this is likely to be a mechanism for the observed alterations in blood electrolytes and the secondary changes in the vitreous [24, 42, 58, 59, 83, 85]. After drowning, the PMVSC initially increases and then remains steady for at least 1 hour prior to further elevating because of immersion effects, at which point the PMVSC level is rendered uninterpretable [58, 59]. This conclusion was based on the observation that bovine eyes immersed in salt water for up to 1 hour did not present an increase in PMVSC, while in SWD deaths with less than 1 hour of immersion, the PMVSC was significantly increased. Due to research and ethical constraints, it would be difficult to perform any prospective rigorous experimental or clinical studies that could confirm that the initial increase in PMVSC in SWD resulted from drowning. This case report was able to support the observation that during SWD, PMVSC initially increased as a result of salt water inhalation and ingestion.
Section 2 Establishing the role of post mortem vitreous magnesium level in the interpretation of vitreous sodium and chloride levels in suspected salt water drowning when the immersion time is greater than 1 hour or is unknown

Section 1 established post mortem vitreous sodium and chloride levels as a useful adjunct in the diagnosis of salt water drowning (SWD). A limitation in the use of vitreous sodium and chloride levels is that the immersion time must be less than 1 hour because after 1 hour, post mortem vitreous sodium and chloride levels increase due to immersion. However, it is common to encounter deaths with an immersion time that is either longer than 1 hour or is unknown. It would be ideal to have a “marker” that was unaffected by SWD and could indicate whether the effects of immersion had occurred. This section contains three studies that established the use of post mortem vitreous magnesium to interpret vitreous sodium chloride levels in suspected SWD.

Like sodium and chloride, magnesium can diffuse through the eye coverings (the cornea, conjunctiva and sclera) during prolonged salt water immersion [54, 55, 75, 84]. In SWD, magnesium is increased in the blood, pleural fluid and pericardial fluid [86-90], suggesting that magnesium can enter the circulatory system. However, magnesium is relatively unable to permeate the intact blood-brain barrier [91, 92] and can only pass through via active transport [93-95]. The blood-ocular barrier consists of non-fenestrated retinal capillaries and tight junctions between retinal epithelial cells [96], similar to the blood-brain barrier, and it can prevent substances from being freely exchanged between blood, retinal tissue and vitreous humour. It is likely that vitreous magnesium would be relatively stable despite acute changes in blood magnesium.

The first study in this section, published in the American Journal of Forensic Medicine and Pathology (Tse R, Kuo TC, Kesha K, Garland J, Garland S, Anne S, Elstub H, Cala A. Post mortem Vitreous Humour Magnesium Does Not Increase in Salt Water Drowning When the Immersion Time Is Less Than an Hour. Am J Forensic Med Pathol. 2017Dec;38(4):298-303), compared vitreous magnesium, sodium and chloride levels between SWD deaths with immersion times of less than 1 hour, SWD deaths with immersion times greater than 1 hour and non-immersion deaths. This study, reproduced in this thesis as chapter 6, showed that in SWD deaths with immersion times of less than 1 hour, vitreous magnesium did not increase,
whereas sodium and chloride did. This study formed the basis of the hypothesis that vitreous magnesium increases due to immersion but not as a result of drowning, making it a potential marker for the effects of immersion.

A subsequent study published in the American Journal of Forensic Medicine and Pathology (Tse R, Kuo TC, Garland J, Lam L, Sunderland M, Kesha K, Elstub H, Cala A, Stables S. Post mortem Vitreous Sodium and chloride Increase After 1 Hour and Magnesium After 2 Hours in Bovine Eyeballs Immersed in Salt Water. Am J Forensic Med Pathol. 2018 Sep;39(3):242-246) compared the relative changes in vitreous sodium, chloride and magnesium levels when bovine eyes were immersed in salt water. This study, reproduced in this thesis as chapter 7, showed that vitreous magnesium increased later than sodium and chloride during salt water immersion. This study concluded that if the immersion time is less than 1 hour, sodium and chloride can be interpreted because the effects of immersion will not have set in. If the immersion time is unknown or greater than 1 hour, vitreous magnesium would be useful for determining whether the effects of immersion had set in: If vitreous magnesium is not increased, vitreous sodium and chloride levels can be interpreted because the effects of immersion should be minimal, whereas if vitreous magnesium is increased, vitreous sodium and chloride levels cannot be interpreted because the effects of immersion will have already begun. The final study, published in the Forensic Science International (Tse R, Garland J, Kesha K, Morrow P, Lam L, Elstub H, Cala AD, Palmiere C, Stables S. Post mortem vitreous magnesium in adult population. Forensic Sci Int. 2018 Mar;284:46-52), reproduced in this thesis as chapter 8, established a normal post mortem vitreous magnesium level in humans, thus providing a reference range for its interpretation. The overall finding of these three studies is that the post mortem vitreous magnesium level can be a useful marker for the effect of salt water immersion. It can be used to interpret vitreous sodium and chloride levels when the immersion time is greater than 1 hour or is unknown.

This section concludes with an illustrative case of a man who died while diving at sea. When his body was recovered, his diving equipment was removed. Due to his body habitus, he could not be retrieved from the water and was pulled to shore by a boat. The duration in which he was immersed in salt water was not clearly documented. Vitreous magnesium was not increased, indicating that vitreous sodium and chloride levels, which were increased, could be interpreted. A diagnosis of SWD was subsequently made based on the vitreous electrolyte analysis.
Chapter 6. Increased post mortem vitreous magnesium levels in salt water downing deaths are from immersion and not drowning


I was involved in initiating, managing and executing this study, which included conceiving the original idea; supervising the study; designing the study; applying for ethical approval; securing funding; collecting data; running the study; writing, reviewing and submitting the manuscript; and subsequently responding to the reviewer’s comments. The study was approved by the Hunter New England Human Research Ethics Committee (Authorization number: AU201602-10).

Section 1 demonstrated that post mortem vitreous sodium and chloride levels (PMVSC, sum of vitreous sodium and chloride levels) are a useful adjunct test in the diagnosis of SWD, especially when the immersion time is less than 1 hour (SWD-1) [57, 59]. The PMVSC increase in SWD-1 is hypothesized to be related to blood electrolyte changes secondary to salt water inhalation/ingestion and is reflected in the post mortem vitreous humour [57, 59].

After sodium and chloride, the third most abundant element in salt water is magnesium. It has a typical concentration of 50-60 mmol/L, which can be up to approximately 50 times higher than the magnesium concentration in the blood and vitreous humour [54, 55, 75, 76]. Like sodium and chloride, magnesium can diffuse through the eye coverings during prolonged salt water immersion [54, 55, 75, 84]. In SWD, magnesium is increased in bodily fluids [86-90], suggesting that it can enter the circulatory system. Interestingly, magnesium is relatively unable to permeate the intact blood-brain barrier [91, 92] and can only pass through via active transport [93-95]. Clinically, the magnesium level in the cerebrospinal fluid remains constant despite the administration of intravenous magnesium or prolonged induced hypermagnesemia [91, 92], and the transport of magnesium through the blood-brain barrier is mostly via slow active transport [93-95]. Similar to the blood-brain barrier, the blood-ocular barrier prevents the free exchange of substances between the blood, retinal tissue and vitreous humour. Thus,
it was expected that vitreous magnesium would be relatively stable despite acute changes in blood magnesium levels.

It was hypothesized that any increase in post mortem vitreous magnesium (PMVM) in cases of salt water drowning (SWD) would result from salt water immersion and not drowning. This study investigated PMVM and PMSC changes in SWD and tested the following hypotheses:

- PMVM levels are higher in SWDs with immersion times greater than 1 hour (SWD>1) than in both SWD-1 and non-immersion deaths.
- There is no difference in PMVM between SWD-1 and non-immersion deaths.
- PMVSC is lower in non-immersion deaths than in both SWD-1 and SWD>1.
- PMVSC is higher in SWD>1 than SWD-1.

6.1. Materials and methods

A 1-year retrospective study (2016) comparing PMVSC and PMVM in non-immersion deaths, SWD-1 and SWD>1 collected from the Department of Forensic Medicine, Newcastle, Forensic & Analytical Science Service (FASS), New South Wales Health Pathology was performed. For all three groups, the age, sex, time between death and the post mortem examination, and sodium, chloride and magnesium levels in the vitreous humour were recorded.

6.1.1. Case selection: SWD-1 and SWD>1

All water immersion-related deaths in 2016 were identified. SWD-1 cases were identified by selecting all clinical and post mortem diagnosed salt water drowning deaths with clear documentation of an immersion time of less than 1 hour in the referring police report. SWD>1 cases were identified in a similar manner except that the immersion times were clearly documented as above 1 hour.

6.1.2. Control selection: Non-immersion deaths

Non-immersion deaths were selected sequentially from cases for which the levels of sodium, chloride and magnesium in the vitreous humour were analysed at post mortem during the same study period in the same department.
6.1.3. Exclusion criteria

Cases in which the immersion time was not documented were excluded. Sudden unexpected infant deaths, suspicious deaths, homicides, and bodies that were decomposed or incinerated or had conditions that may cause electrolyte changes were excluded.

6.1.4. Vitreous electrolyte analysis

All vitreous humour samples were analysed for sodium, chloride and magnesium by a local accredited biochemistry lab (Pathology North, John Hunter Hospital, New Lambton Heights, New South Wales, Australia) using an ion selective electrode and photometric analyser (Abbott chemistry analyser C16000/C8000). All samples were transported in a plain tube without any preservatives. The samples were centrifuged/spun down prior to analysis without heat treatment. In the non-immersion, SWD-1 and SWD>1 cases, the PMVM was recorded, and the PMVSC was calculated by adding the vitreous humour sodium and chloride levels.

6.1.5. Statistical methods

SAS 9.4 (SAS Institute, Cary, North Carolina, USA) was used for analysis.

Continuous variables were described using means and standard deviations. Medians, minima and maxima were also presented. Categorical variables were described using counts and percentages for the non-immersion death, SWD-1, and SWD>1 groups.

The differences between the three groups in terms of age, time between death and the post mortem examination, PMVSC, and PMVM were first assessed for normality and then assessed with either the ANOVA or Kruskal-Wallis test. This was followed by a Student’s t-test and Wilcoxon’s two-sample test. Between-group differences in sex were assessed using the Pearson chi-square test.

6.2. Results

There were a total of 66 immersion-related deaths during the study period; of these, 11 were SWD-1, and 7 were SWD>1. The cases excluded from the analysis were non-SWDs, immersion deaths unrelated to drowning, and cases in which the state of the body was such that the vitreous humour could not be extracted. The locations of the deaths were local beaches along the east coast of Australia facing the Tasman Sea. In the 11 cases of SWD-1, the average age was 49 years (range: 28-78), and all cases were males. The average time
between death and the post mortem examination was 3 days (range: 2-6 days). In the 7 cases of SWD>1, the average age was 50 years (range: 14-88), and there was a male predominance (M:F: 5:2). The average time between death and the post mortem examination was 3.4 days (range: 2-6 days).

A total of 97 non-immersion deaths were identified as suitable for the study. Conditions that were excluded included diabetic ketoacidosis, end-stage liver disease, insulin toxicity, pancreatitis, and hyponatraemia. There were 49 cases of sudden cardiac death from various causes, 23 cases of acute drug and alcohol toxicity-related deaths, 9 cases of pulmonary-related deaths (asthma, pneumonia and pulmonary fibrosis), 6 cases of acute traumatic deaths, 6 cases of acute neurological causes (brain haemorrhage and sudden unexpected death in epilepsy), and 4 miscellaneous causes. The average age was 52 years (range: 24-79) with a male predominance (M:F: 60:37). The average time between death and the post mortem examination was 4.4 days (range: 1-10 days).

### 6.2.1. Demographics and the time between death and the post mortem examination

A summary is presented in table 6.1. There was no difference in age between the three groups. There were significant differences in sex between non-immersion deaths, SWD-1 and SWD>1, with SWD-1 having a higher male predominance.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Level</th>
<th>Non-immersion deaths</th>
<th>SWD-1</th>
<th>SWD&gt;1</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(n=97)</td>
<td>(n=11)</td>
<td>(n=7)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>Female</td>
<td>37 (38%)</td>
<td>0 (0%)</td>
<td>2 (29%)</td>
<td>0.0386</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>60 (62%)</td>
<td>11 (100%)</td>
<td>5 (71%)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>Mean (sd)</td>
<td>52(12.73)</td>
<td>49(19)</td>
<td>50(25)</td>
<td>0.7267</td>
</tr>
<tr>
<td></td>
<td>Median (min, max)</td>
<td>52(24, 79)</td>
<td>53(28, 78)</td>
<td>54(14, 88)</td>
<td></td>
</tr>
<tr>
<td>Days to the post mortem examination(days)</td>
<td>Mean (sd)</td>
<td>4.4(1,74)</td>
<td>3.0(1,41)</td>
<td>3.4(1,51)</td>
<td>0.0108</td>
</tr>
<tr>
<td></td>
<td>Median (min, max)</td>
<td>4(1,10)</td>
<td>2(2, 6)</td>
<td>3(2, 6)</td>
<td></td>
</tr>
</tbody>
</table>

Table 6.1: Summary of the demographic and time data between death and the post mortem examination in cases of non-immersion death, salt water drowning deaths with immersion times less than 1 hour (SWD-1) and salt water drowning deaths with immersion times greater than 1 hour (SWD>1).

There was a significant difference in time between death and the post mortem examination between non-immersion deaths, SWD-1 and SWD>1. The time between death and the post mortem examination was 3 days (range: 2-6 days). In the 7 cases of SWD>1, the average age was 50 years (range: 14-88), and there was a male predominance (M:F: 5:2). The average time between death and the post mortem examination was 3.4 days (range: 2-6 days).

A total of 97 non-immersion deaths were identified as suitable for the study. Conditions that were excluded included diabetic ketoacidosis, end-stage liver disease, insulin toxicity, pancreatitis, and hyponatraemia. There were 49 cases of sudden cardiac death from various causes, 23 cases of acute drug and alcohol toxicity-related deaths, 9 cases of pulmonary-related deaths (asthma, pneumonia and pulmonary fibrosis), 6 cases of acute traumatic deaths, 6 cases of acute neurological causes (brain haemorrhage and sudden unexpected death in epilepsy), and 4 miscellaneous causes. The average age was 52 years (range: 24-79) with a male predominance (M:F: 60:37). The average time between death and the post mortem examination was 4.4 days (range: 1-10 days).
mortem examination was significantly longer in the non-immersion death group compared to
the SWD-1 group (p=0.0069). However, there was no significant difference in non-
immersion deaths compared to SWD>1 (p=0.1274) or SWD-1 compared to SWD>1
(p=0.4414).

6.2.2. PMVSC and PMVM

For the vitreous humour analysis, there were significant differences in PMVM and PMVSC
between non-immersion deaths, SWD-1 and SWD>1 (Table 6.2).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Level</th>
<th>Deaths</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Non-immersion deaths</td>
<td>SWD-1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(n=97)</td>
<td>(n=11)</td>
</tr>
<tr>
<td>PMVSC (mmol/L)</td>
<td>Mean (sd)</td>
<td>231.2(20.99)</td>
<td>275.91(8.25)</td>
</tr>
<tr>
<td></td>
<td>Median (min, max)</td>
<td>233(163, 271)</td>
<td>275(263, 290)</td>
</tr>
<tr>
<td>PMVM (mmol/L)</td>
<td>Mean (sd)</td>
<td>1.22(0.41)</td>
<td>1.27(0.47)</td>
</tr>
<tr>
<td></td>
<td>Median (min, max)</td>
<td>1(1, 2)</td>
<td>1(1, 2)</td>
</tr>
</tbody>
</table>

Table 6.2: Summary of post mortem vitreous sodium chloride (PMVSC) and post
mortem vitreous magnesium (PMVM) levels in non-immersion deaths, salt water
drowning deaths with immersion times less than 1 hour (SWD-1) and salt water
drowning deaths with immersion times greater than 1 hour (SWD>1).

PMVM in the SWD>1 group was significantly higher compared to the non-immersion deaths
(p=0.0006) and SWD-1 groups (p=0.034). There was no significant difference in PMVM
between non-immersion deaths and SWD-1 (p=0.6773) (figure 6.1). The PMVSC levels of
the non-immersion death group were significantly lower than those of the SWD-1 (p<0.0001)
and SWD>1 groups (p<0.0001). There were no significant differences in PMVSC between
the SWD-1 and SWD>1 groups (p=0.3414) (figure 6.2).
Figure 6.1: Post mortem vitreous sodium chloride (PMVM; mmol/L in non-immersion deaths, salt water drowning deaths with immersion times less than 1 hour (SWD-1) and salt water drowning deaths with immersion times greater than 1 hour (SWD>1)

Figure 6.2: Post mortem vitreous magnesium (PMSC; mmol/L) in non-immersion deaths, salt water drowning deaths with immersion times less than 1 hour (SWD-1) and salt water drowning deaths with immersion times greater than 1 hour (SWD>1)

6.3. Discussion

6.3.1. PMVM in salt water drowning

The PMVM level of the SWD>1 group was significantly higher than that of the SWD-1 and non-immersion death groups, and no significant difference was demonstrated between SWD-1 and non-immersion deaths. This finding confirms the hypotheses and agrees with clinical observations [91, 92] and the physiological properties of the eyes [76, 93-96]. Furthermore, it
implies that an increase in PMVM in SWD would result from the passive diffusion of salt water magnesium through the eye coverings during immersion and not from drowning.

6.3.2. PMVSC in salt water drowning

The PMVSC level of the non-immersion death group was significantly lower than those of both the SWD-1 and SWD>1 groups. This finding confirms the hypothesis and agrees with previous studies [57, 59]. Although PMVSC was hypothesized to be higher in the SWD>1 than in the SWD-1 group, the change in the SWD>1 group was only trending and was not significantly higher than that of the SWD-1 group. This result was likely due to the relatively higher increase in PMVSC from salt water drowning compared to immersion, the small sample size of this study and the 1-hour period used to segregate SWD cases.

The most plausible reason that PMVSC was not significantly higher in the SWD>1 group compared to the SWD-1 group was the relatively higher increase in PMVSC from SWD compared to immersion. The increase in PMVSC from immersion would be relatively slower than the increase due to drowning, hence muting its effect.

Regarding sample size, when a deceased individual cannot be recovered quickly from salt water, it is highly likely that he/she will not be recovered until days or weeks later; during that time, decomposition will have set in, rendering vitreous humour testing unfruitful. This was a major limiting factor in obtaining a larger sample size for this study.

The 1 hour period used to segregate the SWD cases into SWD-1 and SWD>1 groups was based on the study presented in chapter 4, which found no changes in PMVSC when bovine eyes were passively immersed in salt water for less than 1 hour [84]. This previous study most likely underestimated the time required for salt water sodium and chloride to diffuse into the vitreous humour through the eye coverings as it used enucleated rather than in situ eyes. PMVSC appeared to show an increasing trend with longer immersion times. In particular, four cases in the SWD>1 group with an immersion time greater or equal to 3 hours had PMVSCs between 302-316 mmol/L, which is higher than any PMVSC in the SWD-1 group (max. = 290 mmol/L).
6.4. Limitations

6.4.1. Sample size and case selection

This study was limited in size. Although our results were significant and in keeping with clinical observations, physical properties of the eyes, and previous studies [57, 59, 76, 84, 91-95], future studies should confirm these findings with larger sample sizes.

6.4.2. Variable electrolyte levels in salt water and vitreous

Similar to the study presented in section 1, the results generated from this study were based on cases retrieved from local salt water in the local catchment area; hence, they have similar limitations. Again, similar to previous chapters, the baseline vitreous electrolyte levels prior to death were assumed to be similar between the groups, although this cannot be confirmed.

6.4.3. Demographics and time between death and the post mortem examination

There was a significant difference in sex between the three study groups, with a higher male predominance in the SWD-1 group. There was no significant difference in age. According to Australian data, men are overrepresented in drowning deaths, and it was not surprising that a significant difference was present [97]. Serum magnesium levels have been documented to decrease with age and are higher in males [98]. The differences were as high as 0.03 mmol/L between the sexes and 0.09 mmol/L among ages between 1-74 years [98]. These variations are very low compared to the variations in PMVM, which had an overall average difference of 1.3 mmol/L in this study. PMVM has been reported to be age dependent and is higher in children; it was almost 3 mmol/L in term infants, then decreased to approximately 1.0 mmol/L among 10-year-old children and stabilized thereafter [78]. This study did not include any subjects below the age of 10 years, and our results cannot be extrapolated to the child/infant population. Overall, the effects of sex and age on PMVM are clinically insignificant in this study provided that the deceased subject is within the presented demographic.

The time between death and the post mortem examination differed significantly among the three study groups, with a mean difference of approximately 1-1.5 days. PMVM and serum magnesium are known to increase during the post mortem period due to the release of magnesium from cell death [55, 88, 99]. PMVM has been described as higher in post mortem specimens compared with clinical reference ranges but is documented to be stable up to 48
hours after death [99]. PMVM increases progressively after death at a very low rate (0.004 mol/L per day) [55]. The increase is again very low in comparison to PMVM, which had an overall average of 1.3 mmol/L in this study. This is probably due to the relatively acellular nature of vitreous humour. The overall contribution of the time between death and the post mortem examination to PMVM was clinically insignificant in this study.

The time between death and the post mortem examination was longer in this study than that reported by other departments and offices. The department from which the data were collected for the study covers a vast area of New South Wales, Australia, and operates within a coronial system. Although not always ideal, delays in performing post mortem examinations of 48 hours or longer after death are not uncommon. The effects of the post mortem interval on PMVSC and PMVM were previously studied, and it appeared that PMVSC decreased and PMVM may increase during the post mortem period [73, 100]. Theoretically, the effects of the post mortem interval would reduce the sensitivity of this study, but its effect on PMVSC and PMVM appears to be small and is overshadowed by the effects of drowning and immersion.

6.4.4. Similar electrolyte diffusion rates for sodium, chloride and magnesium across eye coverings

This study assumed that the effects of passive immersion in salt water on PMVM and PMVSC are similar. A subsequent study, presented in chapter 7, will compare the relative changes in PMVM and PMVSC during salt water immersion.

6.5. Conclusion

This study investigated the changes in PMVM in cases of SWD death. It found that while there was no difference in PMVM between the non-immersion death and SWD-1 groups, PMVM was increased in the SWD>1 group. This finding suggests that PMVM does not increase as a result of SWD but because of the duration of immersion, making it a potential marker for the effects of immersion. The relative changes in PMVSC and PMVM are presented in the following chapter.
Chapter 7. The effect of salt water immersion on post mortem vitreous sodium, chloride and magnesium levels


I was involved in initiating, managing and executing this study, which included conceiving the original idea; co-supervising the study; designing the study; applying for ethical approval; securing funding; running the study; collecting data; analysing the data; writing, reviewing and submitting the manuscript; and, subsequently, responding to the reviewer’s comments. This study was funded by the Royal College of Pathology Australasia (RCPA). This study used commercially available deceased animal parts with approval from the Auckland District Health Board research office (project number A+7627).

Section 1 established post mortem vitreous sodium and chloride levels (PMVSC, the sum of vitreous sodium and chloride levels) as a useful adjunct test in the diagnosis of SWD when the immersion time was less than 1 hour [57, 59]. During SWD, large amounts of salt water are inhaled and/or ingested, causing a rapid shift in electrolytes in the blood/plasma that result in a significant increase in PMVSC [59]. The initial increase in PMVSC from drowning eventually stabilizes, and after 1 hour of immersion, salt water sodium and chloride are able to diffuse across the eye coverings, rendering PMVSC uninterpretable [54, 55, 58, 75].

Another abundant element in salt water is magnesium, which can also diffuse across the eye coverings to the vitreous in cases of prolonged immersion [54, 55, 75]. Compared to PMVSC, post mortem vitreous magnesium does not increase as a result of SWD [83]. This difference in the properties of post mortem vitreous magnesium may be useful in the interpretation of PMVSC in suspected SWD deaths when the immersion time is greater than 1 hour or is unknown. For post mortem vitreous magnesium to be a marker for immersion, the relative changes in vitreous sodium, chloride and magnesium need to be established. This study used bovine eyes as human surrogates, immersing them in salt water for up to 6 hours to compare the changes in vitreous sodium, chloride and magnesium.
7.1. Materials and methods

7.1.1. Case selection

Fresh commercially acquired bovine eyes (deceased for 2-8 hours) were obtained from a local abattoir (Auckland Meat Packaging). The eyelids were removed, but the extraocular adherent soft tissues were left intact. The eyes were divided into two groups (the control and “salt water” groups). The eyes allocated to the “salt water” group were directly immersed in a plastic tank containing 20 L of salt water collected from a local beach (Muriwai Beach on the west coast of Auckland; the water was from the Tasman Sea, New Zealand, and contained sodium: 480 mmol/L, chloride 474 mmol/L, and magnesium: 53 mmol/L). The volume of salt water was chosen to exceed the volume of each bovine eye by more than 95% so that the effect of any electrolyte transfer would be negligible. The temperature of the salt water in the tank was kept steady at between 15 and 25°C. The eyes allocated to the control group were placed inside a sealed, impermeable, dry plastic bag placed next to the water tank.

7.1.2. Vitreous electrolyte analysis

Vitreous humour from each group was sampled at 1 hour, 2 hours, 4 hours and 6 hours by aspirating all the vitreous from each eye (approximately 7-10 ml) with a fresh sterile 16-gauge needle connected to a 20-mL syringe. The samples were placed in a plain container containing no preservatives and/or additives. The samples were analysed for sodium, chloride and magnesium by a local accredited biochemistry lab (Department of Biochemistry, LabPLUS, Auckland City Hospital, Auckland, New Zealand). Prior to analysis, the vitreous aliquots were heat treated (100°C for 5 minutes) according to the local standard protocol [71]. Sodium, chloride and potassium were measured using an ion selective electrode on a Roche Cobas ISE module. Vitreous magnesium was measured on a Roche Cobas C502 using the xylidyl blue method. The typical coefficient variations for sodium, chloride and magnesium in the bovine vitreous matrix were 1.9% (151 mmol/L), 1.5% (120 mmol/L) and 3.7% (0.9 mmol/L), respectively.

Guided by previously published data [57, 58], this study was powered such that a sample of six eyes at each time-point from each group would provide a power of 80% to detect a 10-mmol/L change in vitreous sodium and chloride and a 0.2-mmol/L change in vitreous magnesium at a 5% statistical significance threshold. Six extra bovine eyes were examined at 0 hours to provide baseline vitreous sodium, chloride and magnesium levels.
7.1.3. Statistical methods

The statistical program R v3.4.1 (The R Foundation for statistical computing) was used for the analysis.

Data were summarized at each time-point separately for each group using means, standard deviations and medians with maxima and minima. The differences between the control and “salt water” groups were compared at each post-baseline time-point using permutation tests. For the analysis, we employed resampling statistical analysis methods, which are robust to departures in model assumptions, such as normality and constant variance. Linear regression was used to explore the trends across time in both groups.

7.2. Results

Summary statistics for vitreous sodium, chloride and magnesium at each time-point for the control and “salt water” groups and the between-group differences at each time-point are presented in Table 7.1.

There were no statistically significant differences in the mean vitreous sodium, chloride and magnesium levels between the two groups at 1 hour. Conversely, a clear statistically significant mean difference in vitreous sodium and chloride was evident at 2 hours (sodium: +4.33 mmol/L, p<0.05; chloride: +3.50 mmol/L, p<0.05; “salt water” vs. control) compared to controls sampled at the same time. This difference was not detected in magnesium until 4 hours (+0.17 mmol/L, p<0.05, “salt water” vs. control).

In the control group, there was a small, insignificant decrease in vitreous sodium, chloride and magnesium over time (sodium: -0.079 mmol/L/hr, p=0.7095, R²=0.0050; chloride: -0.098 mmol/L/hr, p=0.6616, R²=0.0069; magnesium: -0.004 mmol/L/hr, p=0.4846, R²=0.0176).
<table>
<thead>
<tr>
<th>Time</th>
<th>Vitreous electrolytes (mmol/L)</th>
<th>Group</th>
<th>Control</th>
<th>Salt Water</th>
<th>Control-Salt water</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 hours</td>
<td>Na</td>
<td>Mean (sd)</td>
<td>145.00</td>
<td>144.33</td>
<td>0.84 p=0.54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Median (range)</td>
<td>(2.08)</td>
<td>(3.82)</td>
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</tr>
<tr>
<td></td>
<td>Cl</td>
<td>Mean (sd)</td>
<td>116.17</td>
<td>118.50</td>
<td>-1.5 p=0.44</td>
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<tr>
<td></td>
<td></td>
<td>Median (range)</td>
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<td>(3.55)</td>
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<tr>
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<td>Mg</td>
<td>Mean (sd)</td>
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<td>0.92</td>
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<td>(0.07)</td>
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</tr>
<tr>
<td>1 hour</td>
<td>Na</td>
<td>Mean (sd)</td>
<td>145.17</td>
<td>144.33</td>
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<td></td>
<td>Median (range)</td>
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<td>(3.82)</td>
<td></td>
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<tr>
<td></td>
<td>Cl</td>
<td>Mean (sd)</td>
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<td>118.00</td>
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<td>(1.00)</td>
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<td>2 hours</td>
<td>Na</td>
<td>Mean (sd)</td>
<td>142.67</td>
<td>147.00</td>
<td>-4.33 p&lt;0.05</td>
</tr>
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<td></td>
<td>Median (range)</td>
<td>(2.21)</td>
<td>(1.29)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cl</td>
<td>Mean (sd)</td>
<td>114.50</td>
<td>118.00</td>
<td>-3.5 p&lt;0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Median (range)</td>
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<td>(1.91)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mg</td>
<td>Mean (sd)</td>
<td>0.93</td>
<td>0.92</td>
<td>0.01 p=1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Median (range)</td>
<td>(0.05)</td>
<td>(0.07)</td>
<td></td>
</tr>
<tr>
<td>4 hours</td>
<td>Na</td>
<td>Mean (sd)</td>
<td>144.50</td>
<td>152.00</td>
<td>-7.33 p&lt;0.05</td>
</tr>
<tr>
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<td></td>
<td>Median (range)</td>
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<td>(1.57)</td>
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</tr>
<tr>
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<td>Cl</td>
<td>Mean (sd)</td>
<td>116.83</td>
<td>123.00</td>
<td>-5.84 p&lt;0.05</td>
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<tr>
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<td>Median (range)</td>
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<td>(2.56)</td>
<td></td>
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<td></td>
<td>Mg</td>
<td>Mean (sd)</td>
<td>0.90</td>
<td>1.05</td>
<td>-0.17 p&lt;0.05</td>
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<tr>
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<td>Median (range)</td>
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<td>(0.12)</td>
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<tr>
<td>6 hours</td>
<td>Na</td>
<td>Mean (sd)</td>
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<td>159.33</td>
<td>-15 p&lt;0.05</td>
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<tr>
<td></td>
<td></td>
<td>Median (range)</td>
<td>(1.91)</td>
<td>(4.07)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cl</td>
<td>Mean (sd)</td>
<td>115.17</td>
<td>132.83</td>
<td>-17.66 p&lt;0.05</td>
</tr>
<tr>
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<td></td>
<td>Median (range)</td>
<td>(1.07)</td>
<td>(6.91)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mg</td>
<td>Mean (sd)</td>
<td>0.92</td>
<td>1.20</td>
<td>-0.28 p&lt;0.05</td>
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<tr>
<td></td>
<td></td>
<td>Median (range)</td>
<td>(0.04)</td>
<td>(0.2)</td>
<td></td>
</tr>
</tbody>
</table>

Table 7.1: Changes in the post mortem vitreous electrolytes (sodium, chloride and magnesium) of bovine eyes when immersed in salt water for less than 6 hours compared to controls

Due to heterogeneity in the rates of change between sodium, chloride and magnesium in the “salt water” group over time, the prediction intervals for this group were estimated separately by time (1 hour or less and longer than 1 hour for sodium and chloride and 2 hours or less and longer than 2 hours for magnesium). Linear regression analysis did not demonstrate a trend in the first hour for vitreous sodium (p < 0.74) or chloride (p < 0.25). This was followed by
significant increases at a rate of 3.1 mmol/L/hr (p < 0.01) for sodium and 3.7 mmol/L/hr (p< 0.01) in chloride in samples collected after 1 hour. In contrast, no significant trend was detected in the first two hours for vitreous magnesium (p=0.48), followed by a rate of increase of 0.07 mmol/L/hr (p<0.01).

7.3. Discussion

This study compared the changes in vitreous sodium, chloride and magnesium when bovine eyes (used as human surrogates) were immersed in salt water. We demonstrated that vitreous sodium and chloride levels were steady when immersed in salt water for up to 1 hour, and vitreous magnesium levels were steady for up to 2 hours. After 2 hours, vitreous sodium, chloride and magnesium increased steadily at rates of approximately 2%, 3% and 3% per hour, respectively (figure 7.1).

Figure 7.1a: Illustration of the changes in post mortem vitreous sodium, chloride (Y-axis, mmol/L; X-axis, hours)

Figure 7.1b: Illustration of the changes in post mortem magnesium during salt water immersion (Y-axis, mmol/L; X-axis, hours)
Salt water immersion appeared to induce an earlier increase in vitreous sodium and chloride levels, whereas changes in vitreous magnesium were not observed until the eyes had been immersed in salt water for more than 2 hours. These changes in vitreous sodium and chloride levels were in keeping with a similar study that showed no increase after 1 hour of immersion [58] and confirmed a previous observation that alterations in vitreous magnesium in both human and bovine eyes occurred following immersion for 2 hours [54, 75]. From this study, we can conclude that when a deceased individual is recovered from salt water, the level of magnesium in the vitreous can assist in establishing the cause of death when cross-referenced with sodium and chloride (Table 7.2). When the levels of magnesium, sodium and chloride are not increased, the cause of death is not drowning. Conversely, drowning is the probable cause of death when sodium and chloride are increased but magnesium is normal, keeping in mind the 1 hour lag difference between electrolytes. When magnesium, sodium and chloride are increased, the vitreous electrolytes cannot be interpreted because the effects of immersion on the vitreous have already commenced.

<table>
<thead>
<tr>
<th>Vitreous electrolytes</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na</td>
<td>Cl</td>
</tr>
<tr>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Increased</td>
<td>Increased</td>
</tr>
<tr>
<td>Increased</td>
<td>Increased</td>
</tr>
</tbody>
</table>

Table 7.2: Interpretation of vitreous electrolytes (sodium, chloride and magnesium)

7.4. Limitations

7.4.1. Use of bovine eyes and the physical properties of the salt water used

The limitations regarding the use of bovine eyes and local salt water were similar to those noted for the study presented in chapter 3. The limitations regarding the use of bovine eyes (with the ocular adnexa removed) in this type of study were discussed in chapter 3 and will not be further elaborated.

The salt water used in this study was collected in New Zealand, which is different from the study described in chapter 3. Although the sodium and chloride levels in the salt water used in the study were lower than the levels in the salt water used in the previous study, they were approximately three times higher than the levels in the vitreous and plasma, and the magnesium level of the salt water was approximately 50 times higher than the levels in the
vitreous and plasma. Extrapolation of the results to salt water with different electrolyte levels should be performed with caution.

Temperature and water movement were not investigated in the study. The salt water temperature in the study was well controlled at 15-25°C, which may not reflect salt water temperatures at different water depths. The role of tides and waves was not modelled in the study. These factors were considered insignificant due to the short duration of immersion.

7.4.2. Normal vitreous magnesium levels

This study used bovine eyes as a human surrogate. The bovine vitreous magnesium levels showed low variability (sd 0.04-0.08 mmol/L). Although a reference range for magnesium has been established in living humans [101], there is currently no established reference range for the post mortem vitreous magnesium, which limits the utility of the findings presented. A post mortem vitreous magnesium reference range is established in the following chapter.

7.5. Conclusion

There was no statistically significant increase in bovine vitreous sodium and chloride levels after immersion in salt water for up to 1 hour or in vitreous magnesium levels after immersion for up to 2 hours. Assuming similar physical properties in humans, an increase in vitreous magnesium in a deceased individual recovered from salt water would imply that the immersion-related increases in vitreous sodium and chloride levels had already occurred. The limiting factors in this study include the assumption that bovine and human eyes have similar physical properties in terms of sodium, chloride, magnesium, and water permeability. A post mortem vitreous magnesium reference value is established in the following chapter.
Chapter 8. Establishing a reference post mortem vitreous magnesium level


I was involved in initiating, managing and executing this study, which included conceiving the original idea; co-supervising the study; designing the study; securing funding; running the study; collecting data; analysing the data; writing, reviewing and submitting the manuscript; and, subsequently, responding to the reviewer’s comments. All cases selected for this study underwent coronial post mortem examinations approved by the coroner. Biochemical investigations were performed as part of the investigation.

Section 1 established post mortem vitreous sodium and chloride levels (PMVSC, the sum of vitreous sodium and chloride levels) as a useful adjunct in the diagnosis of SWD when the immersion time was less than 1 hour. However, deaths in which the immersion time is either longer than 1 hour or unknown are commonly encountered. When the immersion time is longer than 1 hour or unknown, the PMVSC is rendered uninterpretable due to the potential effect of immersion. It would be ideal to utilize a “marker” that indicates that the effect of immersion occurs independent of drowning. Chapters 6 and 7 of this section concluded that post mortem vitreous magnesium only increases as a result of salt water immersion and not from drowning, making it a good marker of immersion to aid in the interpretation of PMVSC. An increased post mortem vitreous magnesium level in a deceased individual recovered from salt water indicates that the effects of immersion have occurred and that PMVSC cannot be interpreted. Conversely, a normal post mortem vitreous magnesium level indicates that the effects of immersion are minimal, and PMVSC can be interpreted. However, there is currently no accepted reference range for post mortem vitreous magnesium levels, and the relationships between post mortem vitreous magnesium other electrolyte levels, disease conditions, age and sex have not been fully established. The study presented in this chapter was conducted to examine the relationships between post mortem vitreous magnesium and age, sex, diabetic state, post mortem interval (PMI) and other electrolytes (sodium, chloride and potassium) and to establish a reference range for post mortem vitreous magnesium levels.)
8.1. Materials and methods

8.1.1. Case selection

To establish a correlation coefficient of 0.5 (moderately associated, with $\alpha=0.05$, $\beta=0.2$), the required sample size ($n$) was calculated as 29. Clinical data showed that the mean difference in vitreous magnesium between diabetic and non-diabetic patients was approximately 0.15 mmol/L, with a standard deviation of 0.15 mmol/L. To achieve $\alpha=0.05$, $\beta=0.2$, a sample size of 19 was needed for each group.

This study subsequently examined 20 consecutive diabetic and 20 non-diabetic adult (age >18 years) deaths between May and October 2017 at the Department of Forensic Pathology, LabPLUS, Auckland City Hospital. All full post mortem examinations performed at the forensic department were authorized by the coroner and included biochemical analysis.

Cases in which the pathologist had sampled vitreous fluid for biochemical analysis (including sodium, chloride, potassium and magnesium) were identified. The case files were reviewed, and the post mortem interval (PMI) was determined as the difference between the time of death and the time of the post mortem examination (in hours). The age, sex, cause of death, PMI, and electrolyte analysis results were recorded. The diabetic status was determined by accessing electronic medical records.

8.1.2. Vitreous electrolyte analysis

Vitreous fluid was collected from both eyes and combined and either placed in a single plain tube for biochemical analysis or split in half (one half used for toxicological analysis and the other for biochemical analysis). The vitreous fluids submitted for biochemical analysis were sent to a local accredited laboratory (Department of Biochemistry, LabPLUS, Auckland City Hospital, Auckland, New Zealand) for analysis. Prior to the analysis, the vitreous samples were heat treated (100°C for 5 minutes) and spun down as per protocol [71].

Sodium, chloride and potassium were measured using an ion selective electrode on a Roche Cobas ISE module. Vitreous magnesium was measured on a Roche Cobas C502 using the xylidyl blue method. A validity study using the xylidyl blue method was performed using bovine vitreous samples. In the validity study, varying volumes of standard magnesium chloride solution (ACS Reagents, Mexico) were added to bovine vitreous specimens (<10% v/v) following heat treatment. Comparisons between the measured levels of magnesium in
the samples were in agreement with the expected level according to calculations (n=20; N=13 separate eyes; figure 8.1).

![Graph showing validation curve for post mortem vitreous magnesium levels using bovine vitreous](image)

Figure 8.1: Validation curve for measuring post mortem vitreous magnesium levels using bovine vitreous

The calculated level was based on the initial level measured by the Roche xylidyl blue method and the volume of magnesium chloride solution added. The typical variability of our method, as measured by the coefficient of variation for magnesium, was 3.7% (0.9 mmol/L, mean level).

8.1.3. Statistical analysis

The statistical program R v3.4.1 (The R Foundation for Statistical Computing) was used for analysis.

Assuming monotonic and non-parametric relationships among vitreous electrolytes, age and PMI, Spearman’s correlation coefficient was determined for continuous variables and post mortem vitreous magnesium levels. For dichotomous relationships (sex and diabetic state), the vitreous magnesium level was compared using a resampling permutation test. Depending on the result of the permutation test, the ROC curve was plotted, and the AUC was determined. The optimal cut-point was determined by minimizing the Euclidean distance to the perfect predictor (a sensitivity of one and a false positive rate of zero).
8.2. Results

In the 40 identified cases, there were 29 natural deaths (22 deaths from cardiovascular causes, 6 due to infection and 1 from gastrointestinal haemorrhage), 8 drug- and alcohol-related deaths, 2 traumatic deaths and 1 case of hypothermia. A summary of the demographic data, PMI and post mortem vitreous electrolytes is shown in table 8.1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Counts (n)</th>
<th>Mean</th>
<th>sd</th>
<th>Median (max, min)</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>M=30, F=10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Diabetic status</td>
<td>Diabetic =20; non-diabetic=20</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Age</td>
<td>-</td>
<td>52.38</td>
<td>16.65</td>
<td>55 (22, 78)</td>
<td>47.05 - 57.70</td>
</tr>
<tr>
<td>Post mortem interval (PMI, hours)</td>
<td>-</td>
<td>30.99</td>
<td>14.35</td>
<td>30(5, 75)</td>
<td>26.40 - 35.57</td>
</tr>
<tr>
<td>Vitreous sodium (mmol/L)</td>
<td>-</td>
<td>146.3</td>
<td>6.92</td>
<td>146.0(133.0, 167.0)</td>
<td>144.09 - 148.51</td>
</tr>
<tr>
<td>Vitreous chloride (mmol/L)</td>
<td>-</td>
<td>115.8</td>
<td>8.11</td>
<td>117.0(99.0, 139.0)</td>
<td>113.23 - 118.42</td>
</tr>
<tr>
<td>Vitreous potassium (mmol/L)</td>
<td>-</td>
<td>11.85</td>
<td>3.04</td>
<td>11.60(6.20, 20.4)</td>
<td>10.87 - 12.82</td>
</tr>
<tr>
<td>Vitreous magnesium (mmol/L)</td>
<td>-</td>
<td>1</td>
<td>0.12</td>
<td>1(0.8, 1.4)</td>
<td>0.96 - 1.04</td>
</tr>
</tbody>
</table>

Table 8.1: Summary of the age, sex, post mortem interval and post mortem vitreous electrolytes

The vitreous magnesium levels did not show any statistically significant correlations with age, PMI, or the measured vitreous electrolytes (table 8.2). The absolute Spearman’s coefficient was between 0.04-0.21, and none of the coefficients reached statistical significance (p>0.05).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Spearman’s coefficient (rho)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>0.04</td>
<td>0.81</td>
</tr>
<tr>
<td>Post mortem interval (PMI, hours)</td>
<td>0.155</td>
<td>0.34</td>
</tr>
<tr>
<td>Vitreous sodium (mmol/L)</td>
<td>-0.21</td>
<td>0.19</td>
</tr>
<tr>
<td>Vitreous chloride (mmol/L)</td>
<td>-0.21</td>
<td>0.2</td>
</tr>
<tr>
<td>Vitreous potassium (mmol/L)</td>
<td>0.17</td>
<td>0.29</td>
</tr>
</tbody>
</table>

Table 8.2: Spearman’s correlations for post mortem vitreous magnesium and post mortem interval (PMI), age and vitreous electrolytes (sodium, chloride, and potassium)

The permutation test (table 8.3) showed that vitreous magnesium levels were higher in the diabetic subjects (p=0.04, figure 8.2), but there was no difference in vitreous magnesium levels between sexes (p=0.92). The AUC of the ROC for post mortem magnesium level and diabetic status was 0.65 (figure 8.3). The optimal post mortem vitreous magnesium cut-point for discriminating the diabetic state was 1.05 mmol/L, which translated to a sensitivity of 0.35 and a specificity of 0.95.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>sd</th>
<th>Median (max, min)</th>
<th>95% confidence interval</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M (n=30)</td>
<td>1.01</td>
<td>0.09</td>
<td>1.0 (0.8, 1.4)</td>
<td>0.96 - 1.05</td>
<td>0.92</td>
</tr>
<tr>
<td>F (n=10)</td>
<td>0.99</td>
<td>0.09</td>
<td>1.0 (0.9, 1.2)</td>
<td>0.92 - 1.06</td>
<td></td>
</tr>
<tr>
<td><strong>Diabetic status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-diabetic (n=20)</td>
<td>0.96</td>
<td>0.07</td>
<td>1.0 (0.8, 1.1)</td>
<td>0.93 - 0.99</td>
<td>0.04</td>
</tr>
<tr>
<td>Diabetic (n=20)</td>
<td>1.04</td>
<td>0.14</td>
<td>1.0 (0.9, 1.4)</td>
<td>0.97 - 1.11</td>
<td></td>
</tr>
</tbody>
</table>

Table 8.3: Differences in post mortem vitreous magnesium between sexes and diabetic status.

Figure 8.2: Post mortem vitreous magnesium levels (mmol/L) of diabetics (Y) and non-diabetics (N).

Figure 8.3: Receiver operating characteristic (ROC) curves for post mortem vitreous magnesium, with diabetic status as the outcome
8.3. Discussion

In the present study, the vitreous magnesium level appeared to be relatively stable during the post mortem period (mean 1.03 mmol/L, 95% CI: 0.98-1.08 mmol/L) for up to 75 hours when the deceased individual was appropriately refrigerated. Post mortem vitreous magnesium levels did not show any significant correlations with age, PMI, or other vitreous electrolyte levels (sodium, chloride and potassium), and there was no demonstrable difference according to sex. Statistically, diabetics appeared to have higher vitreous magnesium levels, with a mean difference of 0.08 mmol/L, but this difference was not useful as a discriminatory test for diabetes (with an AUC of 0.65 on the ROC curve).

8.3.1. Magnesium homeostasis and post mortem reference range

Magnesium has an atomic number of 12 and a mass of 24.32 Daltons. On average, the human body contains approximately one mole of magnesium. It is the fourth most abundant cation in the human body and the second most abundant cation in intracellular fluid [102]. Magnesium is mainly absorbed in the gastrointestinal tract from the oral intake of water and the consumption of magnesium-rich foods, such as cereal, grains, nuts, legumes, fruits, vegetables and meats [103, 104]. Gastrointestinal absorption of magnesium is inversely related to intake and total body magnesium status [102]. In terms of excretion, the kidney is the major excretory organ for magnesium; it excretes approximately 6% of the body’s filtered magnesium per day, mostly at night [105]. Magnesium is an important intracellular ion due to its role as a cofactor in hundreds of enzymatic reactions responsible for energy metabolism, channel regulation, and the cell cycle [102]. In the human body, it exists in three states: ionized (free), complexed to anions and bound to proteins. Only ionized magnesium is physiologically active [102]. Most magnesium is stored in the bones, soft tissue and muscles; less than 1% is present in the blood circulation, providing a normal serum magnesium level of 0.7-1.0 mmol/L [106]. The movement of magnesium to reach equilibrium between tissue pools is relatively slow, and it has a biological half-life between 41-181 days [102, 104]. In blood, only approximately 60% of magnesium is present in ionized form (approximately two-fifths in serum and three-fifths in red blood cells), which means only small amounts of magnesium are available for diffusion into the vitreous after death [102, 104]. After death, the increase in cell membrane permeability and subsequent cell lysis leads to the slow diffusion of magnesium ions from the intracellular space to the extracellular space and their subsequent diffusion into the vitreous humour [55, 88, 99, 100].
This study showed a mean post mortem vitreous magnesium level of 1.03 mmol/L (with a PMI ranging from 5-75 hours). This was higher than the documented clinical serum reference range, the ante mortem vitreous magnesium level (mean 0.90 mmol/L), and the results of a previous post mortem vitreous magnesium study with a PMI of less than 24 hours [100, 101]. This increase was not unexpected when compared with the clinical serum reference level and ante mortem vitreous level due to the changes that occur during the dying process. This study reported a higher level than a previous study that examined vitreous magnesium in cases of death with a PMI of less than 24 hours [100]. This difference can be explained by the longer PMI interval (discussed below) and differences in sample handling between studies. This study analysed most, if not all, of the vitreous fluid collected from each case, which was then heat treated, centrifuged and analysed [71]. The previous study used spun sequential 0.1-mL vitreous samples analysed with an ion selective electrode, whereas the samples in this study were analysed by a xylidyl blue method (photometric method) on different commercially available platforms [100]. Furthermore, the method used in this study was validated and was found to be accurate, with an error of 3.7% at a mean vitreous magnesium level of 0.9 mmol/L. Due to the substantial difference in sampling and analytical techniques, it may be difficult to directly compare post mortem vitreous magnesium results between this study and the previous study [100]. It is most likely that sampling and analytical techniques contributed to the differences between the studies [70, 72, 107].

8.3.2. Relationship between vitreous electrolytes and the post mortem interval

The analysis of vitreous electrolytes (sodium, chloride, and potassium) in the post mortem setting primarily revolves around estimating the time since death and diagnosing possible metabolic disturbances [3, 70, 73, 108]. The most recent and largest study showed that vitreous sodium and chloride levels decreased during the post mortem interval (2.2 mmol/L/day) and appeared to plateau at extreme PMIs. Changes in vitreous potassium levels appear to behave in the opposite manner, increasing at post mortem and then plateauing [73]. The mechanism of these changes was proposed to be the diffusion of sodium and chloride into the surrounding retina and choroid. Intracellular fluid is low in sodium and chloride and has a high potassium level; hence, the opposite changes occur for potassium. These processes eventually reach equilibrium in cases of prolonged PMIs, showing no further changes in vitreous sodium, chloride and potassium levels [73].
Vitreous and serum magnesium levels should theoretically increase during the post mortem period due to release of intracellular magnesium from cell death [55, 88, 99]. Post mortem vitreous magnesium is described as having a higher level in post mortem specimens compared with clinical reference ranges but is documented to be stable up to 48 hours after death [99]. There are conflicting results regarding the effects of PMI on post mortem vitreous magnesium. Some studies describe a progressive increase after death, while others indicate no effects [101, 109]. The most recent study showed a weak correlation between PMI and post mortem vitreous magnesium but disproves the idea that vitreous magnesium is useful for estimating the time of death during the early post mortem period [100]. The conflicting results and low correlation between post mortem vitreous magnesium and PMI are probably due to the overall low level of magnesium in vitreous samples in both the living and deceased and the fact that equilibrium may have been reached in the early PMI. It has been hypothesized that anoxia causing red blood cell lysis at 48-72 hours will increase post mortem vitreous magnesium, but this has not been explored rigorously [110].

This study did not demonstrate any significant correlation between post mortem vitreous magnesium levels and PMI or vitreous sodium, chloride and potassium levels. This study included PMIs greater than 24 hours and showed no correlation up to 75 hours; thus, the hypothesized increase in post mortem vitreous magnesium secondary to the effect of PMI on cell lysis [22] was not observed. This may be due to an overall low level of available magnesium (ionized form) in the vitreous samples, an equilibrium state having been reached during the early PMI, or cadaver refrigeration slowing the cell lysis process. The results suggest that vitreous magnesium does not correlate with PMI when the PMI is less than 75 hours with cadaver refrigeration. Regarding vitreous sodium, chloride and potassium, the lack of a statistically significant correlation among these electrolytes was not unexpected, as vitreous magnesium did not show a statistical correlation with PMI.

8.3.3. Sex and age

In the literature, post mortem vitreous magnesium levels have been described as being higher in the paediatric population, gradually decreasing and stabilizing beyond 10 years of age [78]. Clinically, the serum magnesium level has been reported to decrease with age, with a difference of 0.09 mmol/L (approximately 10% of normal reference value) between the ages of 1-74 years, but no notable difference was observed in adult vitreous samples [98, 101]. Regarding sex, males appear to have higher serum magnesium levels (0.03 mmol/L,
approximately 3% of normal reference value), but again with no demonstrable difference in ante mortem vitreous magnesium [98, 101].

This study did not show any statistical relationship between post mortem vitreous magnesium levels and age or any difference between sexes. The study population was restricted to adults and did not include any cases below the age of 10 years. Thus, it was not unexpected that in the adult population, post mortem vitreous magnesium levels did not correlate with age and sex, agreeing with the clinical literature [78, 101].

8.3.4. Diabetes

Magnesium is involved in glucose haemostasis and metabolism, and hypomagnesaemia is associated with diabetes with poor glycaemic control [111]. Clinical observation has demonstrated that vitreous magnesium levels are significantly lower in diabetic patients [101]; this was contrary to our results, which showed higher post mortem vitreous magnesium levels in diabetics.

During the process of dying, magnesium is released through cell lysis and then diffuses into the vitreous though the blood vessels that supply the orbit [55, 88, 99, 100]. One possible explanation for the increased post mortem vitreous magnesium in diabetics is that the blood vessels supplying the orbit may have underlying diabetic micro-angiopathy, resulting in easier diffusion into the vitreous. Although the present study showed that the vitreous magnesium level was statistically higher in diabetics, it had a very low discriminatory ability in the diagnosis of diabetes. This may be explained by the complex relationship between magnesium and the diabetic state in the ante and post mortem period described above.

8.4. Limitations

8.4.1. Study design

This study was powered to detect a correlation coefficient of 0.5 (moderate correlation). It is plausible that a weaker correlation (0.1-0.3) between post mortem vitreous magnesium levels with age, other post mortem vitreous electrolytes and PMI may exist; however, detecting such a correlation would require a sample size of almost 200. However, even if any significant correlation were found, its application in actual practice may only be very weak. This result was similar to previous studies and observations [100, 109, 112].
This study did not include the paediatric population. As discussed above, paediatric populations below the age of 10 have significantly higher vitreous magnesium, and the results are clearly not applicable [78]. A further limitation is that longer PMI (>72 hours) with no refrigeration was not specifically investigated in the present study.

This study only investigated the difference in post mortem vitreous magnesium levels in one disease process: diabetes. Magnesium, particularly hypomagnesaemia, is associated with multiple other disease conditions, such as renal disease, cardiovascular disease, eclampsia and metabolic syndrome [111, 113]. These conditions were not investigated in this study and may represent confounding factors.

8.4.2. Vitreous electrolyte analysis

This study used a particular method of analysing vitreous magnesium, which was validated at approximately 0.9 mmol/L. Different pre-analytical preparations of vitreous samples yield different results for sodium and potassium, and the same effects on magnesium would not be unexpected [71, 72, 107]. Furthermore, the use of different analytic methods, such as the ion selective electrode and photometric methods, may potentially introduce further discrepancies [72, 107]. Comparisons with previous studies reveal that our post mortem vitreous magnesium levels were significantly higher, which may be accounted for by the use of different analytical methods [100]. This finding suggests that caution is needed when using the results and reference range established in this study.

8.5. Conclusion

This study showed that vitreous magnesium is relatively stable during the post mortem period. There was no relationship between post mortem vitreous magnesium and sex, age, PMI or other vitreous electrolyte levels. A diabetic state appears to result in a higher post mortem vitreous magnesium level, but the difference was small and of limited practical potential. Overall, the post mortem vitreous magnesium level appears to be relatively stable during the post mortem period (mean 1.03 mmol/L, 95% CI: 0.98-1.08 mmol/L) for up to 75 hours. Variation with age (particularly in paediatric groups), PMI, diabetic status (and possibly other disease processes), and varying analytical methods used in different laboratories may need to be taken into consideration when interpreting post mortem vitreous magnesium levels. The reference range for post mortem vitreous magnesium provided in this
study can be used to interpret post mortem vitreous sodium and chloride levels in suspected SWD deaths when the immersion time is greater than 1 hour or unknown.
Chapter 9. Illustrative case report

Previous chapters in this section described the potential use of post mortem vitreous magnesium (PMVM) levels to aid in the interpretation of post mortem vitreous sodium chloride levels (PMVSC) when diagnosing salt water drowning (SWD) when the immersion time is greater than 1 hour or unknown. The following case describes a salt water immersion death with no clear documentation of how long the deceased had been immersed. The post mortem examination revealed evidence of natural disease processes and the presence of illicit drugs, which could have also been potential causes of death. The use of PMVM to assess whether the effects of immersion had occurred and the subsequent correlation with PMVSC enabled a diagnosis of SWD to be made.

In the presented case (case number: 17F694), the post mortem examination was performed by a forensic pathology registrar/trainee under my supervision.

9.1. Case history

A 45-year-old man went SCUBA diving and was subsequently found deceased by his diving partner. The man was found floating unresponsive at the surface without his face mask or respirator on and had white foam around his mouth. The dive time was 20 minutes to a depth of up to 8 metres. The diving partner was unable to pull the unresponsive man onto the boat, and the body was pulled along the side of the boat while a companion piloted the boat to shore. Emergency medical services attended at the shore and pronounced the diver dead, with no attempted resuscitation. The deceased had no known medical history. It was unclear how long he had been immersed in salt water and pulled alongside the boat before reaching shore.

9.2. Post mortem findings

A post mortem examination was performed 19 hours after the reported death. A post mortem CT scan was performed prior to the post mortem examination and showed no evidence of air embolism. External examination revealed minor superficial injuries over the face. The lungs were waterlogged, oedematous and hyperinflated (left: 580 grams, right 720 grams). Pink froth in the airways was noted. The stomach contained a small amount of watery fluids. The heart (510 grams) showed hypertensive changes and mild to moderate coronary artery disease. The remaining organs were within normal limits and did not show any significant macroscopic or microscopic pathology.
9.3. Ancillary tests

Toxicological analysis showed the presence of methamphetamine (1 g/l) and cannabinoids. Biochemical analysis showed combined post mortem vitreous sodium and chloride levels of 332 mmol/L and a magnesium level of 0.9 mmol/L. There was no natural disease that caused hypomagnesaemia.

9.4. Cause of death

Given the circumstance of the deceased being found floating unresponsive on the surface of the water without his face mask while SCUBA diving and the presence (albeit non-specific) of drowning signs in the lungs, SWD was a possible cause of death. However, a cardiac cause of death was also possible as the incident was unwitnessed, and the lung pathology of a cardiac death can overlap with that of a drowning death. Although there was no evidence of methamphetamine use prior to or during diving, it was also a potential cause or contributing factor to death. Methamphetamine can precipitate a cardiac arrhythmia and have a presentation similar to those of cardiac and drowning deaths. The morphological changes at post mortem examination were not useful for differentiating the competing causes of death in this case. As the immersion time was unknown, PMVSC could not be interpreted on its own. PMVM was performed, showing a level of 0.9 mol/L (at the lower spectrum of the normal range in adults), suggesting that PMVSC had been minimally affected by immersion and therefore could be interpreted. The PMVSC was increased at 332 mmol/L, confirming a diagnosis of SWD. A cardiac or drug-related death would not have caused an increase in PMVSC and would only contribute but not caused the death.

9.5. Discussion

This report illustrated the method used to interpret from an increased PMVSC and a normal PMVM in a man who died during SCUBA diving that the death was caused by SWD. In this case, it was unclear how long the deceased had been immersed in salt water, and the effects of immersion on vitreous electrolytes could not be ruled out. PMVM was very useful in this case to show that the overall effects of immersion on PMVSC would be minimal, indicating that PMVSC could be interpreted. The PMVSC of the deceased was 332 mmol/L, which was above the reported cut-off for the diagnosis of SWD using PMVSC (259 mmol/L) [59].
Cases such as the presented one are commonly encountered in forensic practice. The immersion death was unobserved, the immersion time was unknown, and the post mortem examination showed non-specific signs of multiple conflicting causes of death. It is a great challenge for a pathologist to ascribe a cause of death in these types of cases. As illustrated in the presented case, potential accidental/misadventure death (i.e., drowning), natural death (hypertensive heart disease with coronary atherosclerosis), and drug-related death (methamphetamine toxicity) were all possible. The interpretation of both PMVM and PMVSC was valuable to determine the correct cause of death in this case.
Section 3 Combining post mortem vitreous sodium and chloride levels and lung weights in the diagnosis of salt water drowning

The previous two sections showed the utility of post mortem electrolytes as an adjunct in the diagnosis of salt water drowning (SWD). It is hypothesized that the diagnostic certainty of SWD can be improved by combining different tests and signs rather than interpreting each one separately. With this hypothesis in mind, this final section explored the combination of vitreous sodium and chloride levels with an invasive post mortem finding, lung weight, in the diagnosis of SWD.


The second and final study, published in the American Journal of Forensic Medicine and Pathology (Tse R, Garland J, Kesha K, Morrow P, Lam L, Elstub H, Cala A, Spark A, Palmiere C, Stables S. Combining Post mortem Vitreous Sodium and Chloride and Lung-Body Ratio in Aiding the Diagnosing of Saltwater Drowning. Am J Forensic Med Pathol. 2018 Sep;39(3):229-235), compared the diagnostic certainty of vitreous sodium and chloride levels, the lung-body ratio and the combination of both in the diagnosis of SWD (when the immersion time was less than 1 hour). This study, reproduced in this thesis as chapter 11, showed a quantifiable improvement in diagnostic certainty when both findings were used compared to either finding alone.

The overall result of the two studies is that combining post mortem sodium and chloride levels and lung weight (in the form of the lung-body ratio) can increase the diagnostic certainty of SWD. This result can provide the coroner, police, family and pathologist with actual and potential improvement in the diagnostic certainty of SWD deaths when different types of post mortem examination are performed.

This section concludes with two illustrative cases that demonstrate the use of combining vitreous sodium chloride and lung weight in the diagnosis of SWD. The first case was a four-
year-old child who was witnessed falling into the sea while playing on a jetty. The family strongly objected to a full post mortem examination. A lesser post mortem examination was performed, and the biochemical analysis of the vitreous showed increased sodium and chloride levels consistent with SWD. The coroner and family were advised of the potential improvement in diagnostic certainty if a full post mortem examination was performed. However, the coroner and family were satisfied with the certainty of death from SWD based on vitreous electrolyte analysis and contextual evidence. Thus, a full post mortem examination was avoided. The second case was a death involving a 45-year-old man who fell into the sea during a military exercise. A full post mortem examination was performed to provide the highest diagnostic certainty. By combining the vitreous analysis and lung weight (lung-body ratio), a diagnosis of SWD was made with >95% certainty. These two cases highlighted the ease of using vitreous electrolytes combined with lung weight to diagnose SWD.
Chapter 10. Diagnostic certainty of lung weight, lung-heart ratio and lung-body ratio in the diagnosis of drowning


I was involved in initiating, managing and executing this study, which included conceiving the original idea, study design; collecting data; analysing the data; writing, reviewing and submitting the manuscript; and subsequently, responding to the reviewer’s comments. All cases selected for this study underwent coronial post mortem examinations approved by the coroner. The recording of body and organ weights is standard post mortem practice.

An increase in lung weight is a common finding at post mortem in drowning deaths [3]. The increase in lung weight results from the combination of water aspiration, pulmonary alveolar damage and cardiac failure [3, 38, 40]. Although increased lung weight is observed in drowning, it can be caused by other natural and unnatural conditions [35, 36, 38-41, 114, 115]. The use of lung weight in the diagnosis of drowning has had variable success in the past [35, 36, 38-41, 114, 115]. Multiple variations in the use of lung weight to aid the diagnosis of drowning have been used, including lung weight alone, the lung-heart ratio, the lung-body ratio, and the drowning index (the ratio of lung and pleural effusion to spleen weight) [35, 36, 38-40, 114]. The present study was conducted to compare the diagnostic certainty and robustness of three variations in lung weights (lung weight (L), lung-heart ratio (LH) and lung-body ratio (LB)) in the diagnosis of drowning.

10.1. Materials and methods

Based on previous drowning studies regarding organ weights, the sample size (n) required to detect a combined lung difference of 300 g with a standard deviation of 500 g (α=0.05, β=0.2) was calculated to be 44 [38, 40, 114, 115]. This study included 50 consecutive drowning deaths and 50 non-immersion-related deaths from January 1, 2012, to October 31, 2017, from the Department of Forensic Pathology, Auckland City Hospital. A full post mortem examination was performed in each case, and all organ and body weights were
recorded. All full post mortem examinations performed at the forensic department are authorized by the coroner. For both groups, the electronic case files were reviewed. The age, sex, cause of death, heart weight, lung weights (left and right) and body weight were recorded. The lung weight (L) was determined as the sum of both left and right lungs in grams (g). The lung-heart ratio (LH, g/g) was determined as L divided by heart weight (g). The lung-body ratio (LB, g/kg) was determined as L divided by body weight (kg).

10.1.1. Case selection: Drowning deaths

All drowning deaths were identified through our database by searching “drowning” as the cause of death assigned by the pathologist. Salt water and fresh water drowning deaths were not segregated, as previous studies showed no significant difference in lung weights [3].

10.1.2. Control selection: Non-immersion deaths

Consecutive non-immersion deaths were identified. These included all natural deaths, drug- and alcohol-related deaths, asphyxia deaths, hypothermic deaths and non-exsanguination traumatic deaths. These conditions were included in the non-immersion death group because there can be potential non-drowning differential causes of death in bodies retrieved from water.

10.1.3. Exclusion criteria

- Compromised bodies, including incinerated and/or decomposed bodies.
- Any traumatic or natural deaths that had resulted in exsanguination.
- Paediatric deaths (<10 years of age), including infant deaths.
- Post mortem intervals greater than 3 days, as significant decreases in lung weights are noted after 3 days post mortem [39].

10.1.4. Statistical analysis

The statistical program R v3.4.1 (The R Foundation for Statistical Computing) was used for analysis.

Continuous variables were described using means and standard deviations. Medians, minima and maxima were also presented. Categorical variables were described using counts.
To explore the differences between the two groups, a non-paired Kruskal-Wallis test was used to compare age, and Fisher’s exact test was used to compare the differences in sex distribution between each group.

Assuming monotonic and non-parametric relationships, the Spearman’s correlation coefficient (rho) was determined for age and L, LH, and LB. The differences among L, LH and LB and dichotomous variables (sex and drowning) were analysed using a non-paired Kruskal-Wallis test. Receiver operator characteristic (ROC) curves were plotted for L, LH and LB, with drowning as the outcome. The optimal cut-point was determined by minimizing the Euclidean distance to the perfect predictor (i.e., a sensitivity of one and a false positive rate of zero). The AUCs (an indicator of diagnostic certainty) for L, LH and LB were determined, together with the 95% confidence intervals.

10.2. Results

A summary of the drowning and non-immersion death groups is presented in table 10.1. In the non-immersion death group, there were 36 natural deaths (28 cardiovascular-related deaths, five infective- or inflammatory-related deaths and three gastrointestinal-related deaths), eight drug-related deaths (six synthetic cannabis deaths, one mixed prescription drug toxicity death and one methamphetamine toxicity death), two asphyxia-related deaths, two hypothermic deaths, and two traumatic deaths. The mean age of the drowning group was significantly lower than that of the non-immersion deaths group (p=0.001). There was a trend towards a difference in sex, with a higher proportion of males in the drowning group, but the difference was not statistically significant (p=0.086).

<table>
<thead>
<tr>
<th>Non-Immersion deaths</th>
<th>Count</th>
<th>Mean</th>
<th>Median</th>
<th>sd</th>
<th>Max</th>
<th>Min</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>54</td>
<td>57</td>
<td>17</td>
<td>88</td>
<td>22</td>
<td>49.25</td>
<td>58.87</td>
</tr>
<tr>
<td>Sex</td>
<td>36:14 M:F</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung weight (L, g)</td>
<td>1322</td>
<td>1330</td>
<td>417.8</td>
<td>600</td>
<td>2285</td>
<td>1203.7</td>
<td>1441.18</td>
</tr>
<tr>
<td>Lung-heart ratio (LH, g/g)</td>
<td>2.707</td>
<td>2.67</td>
<td>1</td>
<td>5.74</td>
<td>1.33</td>
<td>2.42</td>
<td>2.99</td>
</tr>
<tr>
<td>Lung-body ratio (LB, g/kg)</td>
<td>15.354</td>
<td>13.806</td>
<td>6.54</td>
<td>38</td>
<td>5.18</td>
<td>13.5</td>
<td>17.21</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Drowning deaths</th>
<th>Count</th>
<th>Mean</th>
<th>Median</th>
<th>sd</th>
<th>Max</th>
<th>Min</th>
<th>95% CI</th>
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<tbody>
<tr>
<td>Age (year)</td>
<td>42</td>
<td>41</td>
<td>18</td>
<td>77</td>
<td>13</td>
<td>36.41</td>
<td>46.67</td>
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<tr>
<td>Sex</td>
<td>43:7 M:F</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung weight (L, g)</td>
<td>1484</td>
<td>1408</td>
<td>496.1</td>
<td>2958</td>
<td>584</td>
<td>1342.75</td>
<td>1624.73</td>
</tr>
<tr>
<td>Lung-heart ratio (LH, g/g)</td>
<td>4.14</td>
<td>9.99</td>
<td>1.64</td>
<td>9.62</td>
<td>1.91</td>
<td>3.67</td>
<td>4.6</td>
</tr>
<tr>
<td>Lung-body ratio (LB, g/kg)</td>
<td>20.45</td>
<td>18.23</td>
<td>8.83</td>
<td>44.51</td>
<td>7.09</td>
<td>17.95</td>
<td>22.96</td>
</tr>
</tbody>
</table>

Table 10.1: Summary of drowning deaths and non-immersion deaths
For continuous variables (Table 10.2), Spearman’s correlation showed a statistically significant positive correlation between age and LH (0.279, p<0.05) and no significant correlation between age and L or LB (p>0.05).

<table>
<thead>
<tr>
<th></th>
<th>Spearman’s coefficient (rho)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung weight (L, g)</td>
<td>-0.1385</td>
<td>0.1691</td>
</tr>
<tr>
<td>Lung-heart ratio (LH, g/g)</td>
<td>0.279</td>
<td>0.004</td>
</tr>
<tr>
<td>Lung-body ratio (LB, g/kg)</td>
<td>-0.0139</td>
<td>0.752</td>
</tr>
</tbody>
</table>

Table 10.2: Spearman’s correlation (rho) between age and lung weight (L), lung-heart ratio (LH, g/g), and lung-body ratio (LB, g/kg)

In terms of the dichotomous variables (Table 10.3), males had a statistically higher L (p<0.05) but no significant difference in LH and LB (p>0.05). Drowning deaths had significantly higher LH and LB (p<0.05), but there was no significant difference in L (p=0.15).

<table>
<thead>
<tr>
<th></th>
<th>P</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Drowning</td>
<td>Male sex</td>
</tr>
<tr>
<td>Lung weight (L, g)</td>
<td>0.15</td>
<td>0.02</td>
</tr>
<tr>
<td>Lung-heart ratio (LH, g/g)</td>
<td>&lt;0.05</td>
<td>0.68</td>
</tr>
<tr>
<td>Lung-body ratio (LB, g/kg)</td>
<td>&lt;0.05</td>
<td>0.4252</td>
</tr>
</tbody>
</table>

Table 10.3: Differences in the male sex and drowning groups in terms of lung weight (L), lung-heart ratio (LH, g/g), and lung-body ratio (LB, g/kg)

The AUCs of the constructed ROC curves were 0.584, 0.787 and 0.678 for L, LH, and LB, respectively (figure 10.1, table 10.4). The optimal cut-points for L, LH and LB were 1290 g (sensitivity: 0.62, specificity: 0.48), 2.99 (sensitivity: 0.78, specificity: 0.48) and 17.26 g/kg (sensitivity: 0.6, specificity: 0.7), respectively.
Figure 10.1a: Receiver operator characteristic (ROC) curves for lung weight (L, g).

Figure 10.1b: Receiver operator characteristic (ROC) curves for lung-heart ratio (LH, g/g).

Figure 10.1c: Receiver operator characteristic (ROC) curves for lung-body ratio (LB, g/kg).

<table>
<thead>
<tr>
<th></th>
<th>AUC</th>
<th>95% CI</th>
<th>Cut-point</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung weight (L, g)</td>
<td>0.58</td>
<td>0.47</td>
<td>0.7</td>
<td>1290</td>
<td>0.62</td>
</tr>
<tr>
<td>Lung-heart ratio (LH, g/g)</td>
<td>0.79</td>
<td>0.7</td>
<td>0.88</td>
<td>2.99</td>
<td>0.78</td>
</tr>
<tr>
<td>Lung-body ratio (LB, g/kg)</td>
<td>0.68</td>
<td>0.57</td>
<td>0.78</td>
<td>17.26</td>
<td>0.6</td>
</tr>
</tbody>
</table>

Table 10.4: Area under the curve (AUC) and corresponding optimal cut-points on the plotted receiver operator characteristic (ROC) curves for lung weight (L), lung-heart ratio (LH) and lung-body ratio, with drowning as the outcome.

10.3. Discussion

10.3.1. Robustness of L, LH and LB with respect to sex and age

Organ and body weights are known to vary among ages and between sexes [3, 116-119], which limits the utility of lung weight on its own in the diagnosis of drowning. Previous
studies used different adjustment methods to account for the variations in age and sex; of these adjustments, LH and LB weight ratios seemed to be most accepted [35, 36, 38-40, 114].

In the present study, the overall age was lower in drowning deaths than non-immersion deaths, with no difference in sex distribution. The effects on the differences between age and sex on the L, LH and LB were explored using Spearman’s coefficient (rho) for continuous variables and non-paired Kruskal-Wallis for dichotomous variables. Overall, L was higher among males and did not correlate with age. LH did not show any difference between sexes but had a positive but weak correlation with age (rho <0.3). LB did not show any significant difference between sexes and had no correlation with age, making it the most robust measurement with age and sex among the three parameters (L, LH, LB). This finding was in keeping with previous studies [38, 40].

10.3.2. Diagnostic certainty

Previous studies have shown that L, LH and LB are statistically higher in drowning deaths compared to non-drowning deaths but were reserved regarding the utility of these measures as reliable diagnostic tests [38, 40]. Although a trend in L was found, this study only showed a statistically significant difference in LH and LB, which was in keeping with previous studies [3, 38, 40, 41, 120, 121]. Furthermore, L, LH and LB and their respective ranges in this study were comparable to those reported in previous studies [38, 40].

In the present study, the diagnostic certainty, assessed using AUCs on the plotted ROC curves, showed that LH had the highest diagnostic certainty, followed by LB and L. The 95% confidence interval for the AUCs of LH and LB were above 0.5 and overlapped, and the superiority of LH vs. LB could not be statistically determined. The AUC determined that L had a 95% confidence interval of falling between 0.47 and 0.70, making it unable to discriminate drowning better than chance.

Based on the robustness of the L, LH and LB with respect to age and sex and the diagnostic certainty assessed by AUC, the most accurate and robust diagnostic parameter was LB. This finding was consistent with previous observations supporting the use of LB over LH and L [38]. The cut-point determined in this study for LB was also in keeping with a previous study [38].

Although this study confirmed previous studies and indicated that LB should be assessed in drowning deaths rather than L and LH [38], its utility as a diagnostic test may be limited. The
diagnostic certainty of LB, which has an AUC of <0.8, in the diagnosis of drowning was ranked as fair and was less accurate than other commonly accepted medical tests. Compared with clinical medical tests, which have an AUC >0.8, LB is less diagnostically accurate [122-124]. This study supports the idea that there is some use in assessing LB, rather than L or LH, in suspected drowning deaths, but its ability to accurately diagnose drowning is at best fair, and it should not be the sole discriminator when diagnosing drowning [38].

10.4. Limitations

10.4.1. Study design

The study examined the diagnostic certainty of lung weight and its variations in differentiating drowning deaths from non-immersion deaths. This study did not separate fresh water drownings and SWDs and did not segregate sex or stratify different age groups. Non-immersion deaths were used as controls and were not further subcategorized into different causes of death.

Although both types of drowning deaths (salt and fresh water) have been shown to cause similar increases in lung weights, extrapolation of our results to fresh water drowning deaths must be performed with caution. This study did not include paediatric populations, especially those below the age of 10 years, and extrapolation beyond these parameters requires great caution. The effects of age and sex on L, LB and LH were examined using correlation studies for continuous variables and Kruskal-Wallis tests for dichotomous variables to determine the robustness of these lung measurements. The results indicated that LB was the most robust and accurate measurement for diagnosing drowning, although it was still poor as a marker on its own.

In terms of control groups, deaths that were not due to drowning but were recovered from water would be the ideal group. These types of deaths are rare, and most would have been excluded in this study. Our previous experience includes approximately 10 cases over an approximately 10-year period [57, 59]. These cases were commonly the result of trauma related to disruption of the body, and accurate organ and body weights cannot be established in such cases. Non-immersion deaths provided a reasonable alternative control group for the purpose of this study. However, in using non-immersion deaths as a control group, this study assumed that lung weight, heart weight and body weight are unaffected by immersion, which is reasonable. It is under this assumption that the results of this study could be applied to
differentiate drowning deaths from deaths that were not due to drowning but in which the deceased was recovered from water.

Subcategorization of non-immersion deaths was not performed because this study was not designed to discriminate drowning from other specific causes of death. However, the non-immersion groups had L, LH and LB values that were not dissimilar to those found in similar studies, and it is our opinion that the non-immersion group was a robust comparison group for drowning for the purpose of this study [38-41].

10.4.2. Confounding factors

Underlying natural diseases and the possible role of intoxication in the drowning deaths used in this study were not recorded or accounted for. It is possible that drowning deaths were overdiagnosed in these cases and that the deaths were in fact due to natural causes and/or intoxication. Not accounting for these conditions in the drowning groups may have resulted in a more robust result and an underestimation of the diagnostic ability of lung weights. Another possible confounding factor was the presence of resuscitation, which was not recorded in this study. Resuscitation may alter the intravascular volume and possibly the lung weight, which may need to be considered.

10.4.3. Optimal cut-points

This study was not designed to determine optimal cut-points for L, LH and LB in drowning deaths. An optimal cut-point can be determined by optimizing both sensitivity and specificity or both with different mathematical techniques, which may yield different optimal cut-points with the same data set. This study optimized both sensitivity and specificity by minimizing the Euclidean distance to the perfect predictor and established a cut-point that was similar to the previously proposed cut-off point for LB [38]. The cut-points determined in the presented study can be used as a reference only.

10.5. Conclusion

Among lung weight (L), lung-heart ratio (LH), and lung-body ratio (LB), LB was the most accurate and robust parameter for the diagnosis of drowning. The accuracy of LB was low, limiting its usefulness as the sole discriminator for drowning (AUC <0.8). Overall, it is recommended that pathologists use LB as a general guide for making a diagnosis of drowning. LB should be interpreted in conjunction with the contextual evidence and other
signs of drowning at post mortem. When there is any evidence of decomposition or an absence of any signs of drowning, LB should be interpreted with caution. Further studies combining LB with other signs or ancillary tests of drowning, such as vitreous electrolytes, may have an additive effect for increasing the diagnostic certainty of drowning. This possibility is explored in chapter 11.
Chapter 11. Combining lung-body ratio and post mortem vitreous sodium and chloride levels in the diagnosis of salt water drowning


I was involved in initiating, managing and executing this study. This included conceiving the original idea; co-supervising the study; designing the study; applying for ethical approval; securing funding; running the study; writing, reviewing and submitting the manuscript; and subsequently, responding to the reviewer’s comments. All cases selected for this study underwent coronial post mortem examination approved by the coroner. Body and organ weight measurement and biochemical analysis were part of the investigation.

Section 1 demonstrated post mortem vitreous sodium and chloride levels (PMVSC, sum of vitreous sodium and chloride levels) as a useful adjunct in the diagnosis of salt water drowning when the immersion time is less than 1 hour (SWD-1). Chapter 10 and previous studies recognized that the lung-body ratio (LB) was the most robust and accurate lung measurement in the diagnosis of drowning [35, 36, 38-40, 114]. It is very possible that combining both PMVSC and LB may improve the diagnostic certainty of SWD-1. This study was subsequently carried out to quantify and compare the performance of LB alone, PMVSC alone and combining PMVSC and LB in the diagnosis of SWD-1.

11.1. Materials and methods

11.1.1. Case selection: SWD-1

All SWD-11 cases were collected from two centres spanning a 5-year period (Department of Forensic Medicine, Department of Forensic Medicine, Newcastle, Forensic & Analytical Science Service (FASS), NSW Health Pathology, John Hunter Hospital, Newcastle, New South Wales, Australia, and Department of Forensic Pathology, Auckland City Hospital, Auckland, New Zealand). SWD-1 cases were identified by selecting all salt water drowning
deaths in which there was a clear diagnosis of salt water drowning as the primary cause of death and documentation of immersion time of less than 1 hour in the referring police report. The case group used in this study was collected from previous studies presented in the previous sections and chapter 10 [59, 83, 125].

11.1.2. Control selection: Non-immersion deaths

For the control group, consecutive non-immersion deaths collected during a one-year period from the Department of Forensic Pathology, Auckland City Hospital, Auckland, New Zealand were identified. This group included all natural deaths, drug- and alcohol-related deaths, asphyxia deaths, hypothermic deaths and non-exsanguination traumatic deaths. These conditions were included in the non-immersion death group because they can be potential non-drowning differentials in bodies retrieved from water. The control group used in this study was the same as a chapter 10 [125].

For both groups, the electronic post mortem files were reviewed. Age, sex, cause of death, heart weight, lung weight (left and right), body weight, and vitreous electrolytes were recorded.

11.1.3. Exclusion criteria

- Immersion deaths in which the pathologist was uncertain the death was from drowning.
- Compromised bodies include incinerated, decomposed bodies and/or bodies with missing body parts.
- All homicidal deaths due to potential legal issues.
- A post mortem interval greater than 3 days, as a significant decrease in lung weights, was noted after 3 days post mortem [39, 40].
- Any cases with incomplete data set.
- All infant and paediatric deaths (less than 10 years old).

11.1.4. Lung-body ratio calculation

For both groups, the lung (left and right) and body weights were recorded from reviewing the electronic medical record. The lung weight was determined as the sum of both left and right lungs in grams (g). The LB ratio (g/kg) was calculated as lung weight divided by body weight (kg).
11.1.5. Vitreous electrolyte analysis

The vitreous humour samples collected from both groups were from the two different departments and analysed at different biochemical laboratories. All samples were placed into a plain container containing no preservatives or additives prior to analysis. PMVSC was calculated by adding vitreous humour sodium and chloride levels.

Vitreous humour samples from SWD-1 collected in Newcastle, Australia were analysed by a local accredited biochemistry lab in Australia (Pathology North, John Hunter Hospital, New Lambton Heights, New South Wales, Australia). The samples were centrifuged/spun down prior to analysis and were not heat treated. Sodium and chloride were measured using an ion selective electrode analyser (Abbott chemistry analyser C16000/C8000).

Vitreous humour samples from SWD-1 and control groups from Auckland, New Zealand were also analysed by a local accredited biochemistry lab in New Zealand (Department of Biochemistry, LabPLUS, Auckland City Hospital, Auckland, New Zealand). Prior to analysis, the vitreous samples were heat treated (100°C for 5 min) and centrifuged. Na and Cl were measured by an ion selective electrode analyser (Cobas ISE module, Roche Diagnostics).

To account for the inter-lab difference, 50 non-immersion deaths from each centre were used to calculate the corresponding z-score for SWD-1 and controls. The z-scores of PMVSCs in both groups were subsequently normalized to centrifuged sample ranges.

11.1.6. Statistical analysis

Statistical program R v3.4.1 (The R Foundation for statistical computing) was used for analysis.

Previous studies showed a difference of approximately 6 g/kg in LB with a standard deviation of 6 g/kg between drowning and non-drowning deaths [38, 125]. This parameter was arbitrarily chosen for this study, where a sample size of less than 20 is required (α=0.05, β=0.2).

In terms of PMVSC, a previous study showed a difference of 40 mmol/L with a standard deviation of 18 mmol/L between SWD-1 and non-drowning deaths [58, 59]. This parameter was arbitrarily chosen for this study, where a sample size of less than 10 is required (α=0.05, β=0.2).
Continuous variables were described using means and standard deviations. Medians, minima and maxima were also presented. Categorical variables were described using counts.

**Between-group variations:**

To explore the differences between the case and control, a non-paired Kruskal-Wallis test was used to compare age, and Fisher’s exact test was used to compare the differences in sex distribution between each group.

**Assessing interactions:**

Assuming monotonic and non-parametric relationships, Spearman’s correlation coefficient (rho) was determined between all continuous variables (age, LB and PMVSC). The differences in LB and PMVSC between sexes were analysed using a non-paired Kruskal-Wallis test.

**Determining diagnostic certainty of LB and PMVSC**

The differences in LB and PMVSC between SWD-1 and control were analysed using a non-paired Kruskal-Wallis test. Receiver operator characteristic (ROC) curves were plotted for LB and PMVSC with SWD-1 as the outcome. The AUC (area under the curve), an indicator of diagnostic certainty, for PMVSC and LB were determined together with the 95% confidence intervals. The optimal cut-point was determined by minimizing the Euclidean distance to the perfect predictor (sensitivity of one and false positive rate of zero).

**Quantifying the diagnostic certainty by combining LB and PMVSC**

Classification tree models were used to quantify and compare the performances of LB, PMVSC and the combination of the two in the diagnosis of SWD-1. Tree models are simple and useful for interpretation. It involves segmenting the predictor space into simple regions by building splitting rules that can be summarized in a decision tree.

As PMVSC and LB were single predictors for SWD-1, one node classification tree model was constructed, which was similar to determining optimal cut-points after constructing ROC curves. For combined LB and PMVSC, statistical learning methods were used to fit the optimal decision tree model. Initially, the recursive binary splitting method was used to expand the decision tree as large as possible. This would, however, cause overfitting and an increase in variance. The expanded decision tree was subsequently pruned down using the
cost complexity pruning method, which minimizes the turning parameter. The misclassification rate, an estimation of test error in statistical learning methods, was determined using K-fold cross-validation for the constructed tree models.

11.2. Results

A total of 35 SWD-1 was identified, with 15 cases excluded for analysis (n=20). The reasons for excluding the cases were due to incomplete dataset, an external only examination was performed, having a post mortem interval of greater than 3 days or the pathologist was not confident the diagnosis was drowning (i.e., immersion death but unsure whether it was drowning). The case files were reviewed, and the context was all accidents during water activity (swimming, diving, and surfing).

In the non-immersion death group, there were 36 natural deaths (28 cardiovascular-related deaths, five infective/inflammatory-related deaths and three gastrointestinal-related deaths), eight drug-related deaths (six synthetic cannabis deaths, one mixed prescription drug toxicity death and one methamphetamine toxicity death), two asphyxia-related deaths, two hypothermic deaths, and two traumatic deaths. There was no significant difference in age and sex between SWD-1 and control (p= 0.13- 0.80). A summary of the SWD-1 and control groups is presented in table 11.1.

<table>
<thead>
<tr>
<th>SWD-1 (n=20)</th>
<th>Age (years)</th>
<th>Mean (sd)</th>
<th>56.2 (14.74)</th>
<th>Median (max, min)</th>
<th>48.5(78, 28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>M:F</td>
<td></td>
<td>18:02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LB (g/kg)</td>
<td>Mean (sd)</td>
<td>18.8(4.82)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung weight (kg)</td>
<td>Mean (sd)</td>
<td>1547.35 (350.01)</td>
<td>1515(2230, 830)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PMVSC (mmol/L)</td>
<td>Mean (sd)</td>
<td>278.61(17.05)</td>
<td>279.61(337.61, 297)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Control (n=50)</th>
<th>Age (years)</th>
<th>Mean (sd)</th>
<th>54.18(16.58)</th>
<th>Median (max, min)</th>
<th>57(88, 22)</th>
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</thead>
<tbody>
<tr>
<td>Sex</td>
<td>M:F</td>
<td></td>
<td>36:14:00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LB (g/kg)</td>
<td>Mean (sd)</td>
<td>15.35(6.54)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung weight (kg)</td>
<td>Mean (sd)</td>
<td>13.83(5.18)</td>
<td>1330(2285, 600)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PMVSC</td>
<td>Mean (sd)</td>
<td>236.19(20.24)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 11.1: Summary of salt water drowning deaths with immersion times of less than 1-hour (SWD-1) and controls.

11.2.1. Assessing interactions

For continuous variables, Spearman’s correlation (rho) showed weak correlations with no significance between age, PMVSC and LB (rho = -0.26-0.11, p=0.27-0.35). There was no significant difference demonstrated in PMVSC and LB between sexes (p=0.13-0.72).

11.2.2. Determining diagnostic certainty of LB and PMVSC

PMVSC and LB were significantly higher in SWD-1 than in the control (p=0.025 for PMVSC, p=0.04 for LB). The AUCs for LB and PMVSC are presented in figure 11.1a and 11.1b. The optimal cut-points for PMVSC and LB were 258 mmol/L (sensitivity: 0.95, specificity: 0.94) and 14.85 g/kg (sensitivity: 0.80, specificity: 0.56), respectively. The AUCs in the constructed ROC curves were 0.949 (95% confidence interval: 0.89-0.99) and 0.683 (95% confidence interval: 0.355-0.81) for PMVSC and LB, respectively. The confidence interval did not overlap between PMVSC and LB.

Figure 11.1a: Receiver-operator-characteristic (ROC) curves for lung-body (LB, g/kg).
11.2.3. Quantifying the diagnostic improvement by combining LB and PMVSC

Decision trees for PMVSC, LB and combined PMVSC and LB were constructed. Graphical summaries of the decision points for PMVSC, LB and combined PMVSC and LB are shown in a scatter plot (figure 11.2a-d). For PMVSC and LB, the decision points were 258 mmol/L and 14.32 g/kg, respectively. For PMVSC, this translates to a value greater than 258 mmol/L supportive of SWD-1 (figure 11.2b). For LB, a value greater than 14.32 g/kg was supportive of SWD-1 (figure 11.2c). When combining PMVSC and LB, the constructed decision tree model showed an initial splitting node for PMVSC at 258 mmol/L and a second-level splitting node for LB at 12.72 (figure 11.2d, and 11.3).

The misclassification rate using K-fold cross-validation in estimating the test errors was 10%, 24% and 4% for PMVSC, LB and combined PMVSC and LB, respectively.
Figure 11.2a: Scatter plot of post mortem vitreous sodium and chloride levels (PMVSC, mmol/L) and lung-body ratio (LB, g/kg).

Figure 11.2b: PMVSC decision point for single predictor (solid line).
Figure 11.2c: LB decision point for single predictor (solid line).

LB=14.32g/kg

Figure 11.2d: Combined PMVSC and LB decision points (solid line-PMVSC, dashed line-LB)

PMVSC=258mmol/L

LB=12.72g/kg
11.3. Discussion

The present study quantified the performance of PMVSC, LB and combined PMVSC and LB in the diagnosis of SWD-1. The combined PMVSC and LB had the highest diagnostic certainty, with the lowest misclassification error followed by PMVSC and LB.

Previous studies have separately reported that PMVSC and LB were statistically higher in SWD deaths [38, 57, 59]. This study again showed that PMVSC and LB were statistically higher in SWD-1, which was not unexpected. The diagnostic certainty, assessed using the misclassification rate from constructing a decision tree model, showed a higher misclassification rate in LB (24%) compared to PMVSC (10%). This was in keeping with previously reported results examining PMVSC and LB in the diagnosis of SWD [57-59, 83]. When combining PMVSC and LB, the first decision node was PMVSC. This demonstrated that PMVSC was more accurate and effective in segregating SWD-1 from control. The second decision node was LB, after which a further segregation of SWD-1 and control was made. The misclassification rate was substantially improved to 4% when these two predictors were used. This was not unexpected, as an addition of useful predictors would logically give better diagnostic certainty.
Combined PMVSC and LB was able to improve the diagnostic certainty of SWD-1 because it was able to discriminate control cases with high PMVSC and low LB and cases with high LB and low PMVSC from SWD-1 (having both high PMVSC and high LB). Cases with high PMVSC and low LB in the control group were individuals with significant physical and mental conditions, which probably resulted in low oral intake, dehydration, and an increased PMVSC but subsequently died from non-immersion causes. These cases were better discriminated using LB rather than using PMVSC. Cases with high LB and low PMVSC in the control group were drug and alcoholic deaths, mainly from alcohol and central nervous system depressants. These cases had higher LB due to respiratory centre depression and had no reason for any increase in PMVSC. These cases were better discriminated using PMVSC rather than using LB. Thus, by combining both PMVSC and LB, the diagnostic certainty would logically increase.

The application of the results generated from this study is relatively simple after accounting for inter-lab differences in PMVSC. In a case of suspected SWD-1 and only PMVSC, the pathologist can use the proposed cut-point of 258 mmol/L in the diagnosis of SWD-1, which would be incorrect 10% of the time. When only LB was available, using a cut-point of 14.32 g/kg would be incorrect 24% of the time. Similarly, if both PMVSC and LB were considered and using the provided decision tree diagram (figure 11.2), an improvement of diagnostic certainty would be reached in which the diagnosis would be incorrect in only 4% of cases.

11.4. Limitations

11.4.1. Variable electrolyte levels in salt water and vitreous

Similar to studies presented in previous chapters in sections 1 and 2, the results generated from this study were based on cases retrieved from local salt water from local catchment areas, hence having similar limitations. Again, similar to previous chapters, the baseline vitreous electrolyte levels prior to death are assumed to be similar between the groups, although this cannot ever be confirmed.

11.4.2. The less than 1-hour time period of immersion

There is a significant number of SWD deaths in which the immersion time is greater than 1 hour or unknown. This study was limited to deceased individuals with an immersion time of less than 1 hour. This chosen time period was due to the limitation in the interpretation of
PMVSC based on previous studies presented in section 1. If the immersion time is greater than 1 hour or unknown, using the results in studies presented in section 2, post mortem vitreous magnesium can be used to determine whether PMVSC can be interpreted.

11.4.3. Case and control selection

Similar to studies presented in the previous sections, this study used non-immersion deaths as the control group. Ideally, cases in which the death was not due to drowning but immersed in salt water for less than 1 hour would be the perfect control group. Based on the results in section 1, using non-immersion deaths as a control for studying SWD-1 was considered reasonable.

The cut-points for PMVSC and LB determined in this study were slightly lower but comparable to previous studies [38, 57, 59]. This may be explained by different post mortem practices between pathologists, different control groups and inter-lab variance.

11.4.4. Applicability of results

Although PMVSC can assist in the diagnosis of SWD death, the general applicability requires adjustments to account for inter-lab differences. Different pre-analytical preparations of vitreous samples can give rise to different results and must be taken account for [71, 72, 107]. The presented study was corrected to spun down vitreous samples without any heat treatment prior to analysis. Before using the presented tree model, PMVSC should be adjusted locally to account for analytical variability between laboratories. Furthermore, PMVSC also only applies to SWD and is not validated for fresh water drowning, which requires further study.

The presented study used a decision tree model for statistical modelling. There are many different statistical methods to classify problems. As illustrated in figure 1a, a statistical model aims to segregate SWD-1 and non-immersion deaths. This can be done with basic classification methods (such as logistic regression, linear discriminant analysis or K-nearest neighbour) or sophisticated methods (such as non-linear models and supportive vector machines). These alternative models could have been used in this study and may possibly improve the diagnostic certainty based on the method used. The decision tree method was chosen in this study because this type of statistical modelling is less abstract, comparatively simple and easy to interpret, and mimics the normal decision-making process in humans. The misclassification rate in the decision tree model was below 5%, and further significant
improvement using other statistical modelling techniques may only have minor improvements.

11.5. Conclusion

This study was carried out to quantify and compare the performance of PMVSC alone, LB alone and combining PMVSC and LB in predicting SWD-1. Using decision tree models, it showed that combining PMVSC and LB had the lowest misclassification rate, followed by PMVSC and LB, in which a substantial improvement was observed when both predictors were used. The decision tree model is simple to use and has a misclassification rate of 4%, making it a reliable method to diagnose SWD-1. This study also highlighted the value of combining different predictors to further improve the diagnostic certainty in a quantitative way.
Chapter 12. Illustrative case reports

The section will present two illustrative cases in which the results of this thesis aided in the diagnosis of salt water drowning (SWD). The first was a paediatric case in which the child was witnessed falling into rough seas while playing on a jetty, and a lesser post mortem examination was performed due to the family’s objection to a full post mortem examination. A diagnosis of SWD was supported by vitreous biochemical analysis results, and a full post mortem examination was not needed. The second case was a military-related death in which the deceased fell into the sea during a military exercise. A very high level of certainty was required due to the high profile nature of the case and the implications of the cause of death. A diagnosis of SWD with high certainty was made based on the vitreous biochemical analysis and lung-body ratio. Unnecessary medical investigation was avoided; a follow-up military investigation was performed, and insurance compensation was provided to the family.

Diagnosing salt water drowning based on increased post mortem vitreous sodium and chloride levels in the context of family objection

This case was a child who was witnessed falling into the sea while playing on a jetty. Two members of the public attempted to rescue the child without success, and the deceased was subsequently rescued by the coast guard staff. The family strongly objected to a full post mortem examination as they felt the cause of death was apparent. The contextual evidence strongly pointed towards SWD, and vitreous biochemistry was able confirm this diagnosis without a full post mortem examination.

In this case (case number: 17F0838), the post mortem examination was performed by a forensic pathologist at Auckland City Hospital, and I was consulted on the post mortem vitreous biochemistry interpretation.

12.1. Case history

A 5-year-old twin was witnessed falling into the sea. The deceased was playing on a jetty with his father and his twin. It appeared that when his father looked away, the child ran on the
jetty and fell into the sea. This event was captured on CCTV and by members of the public. Within a minute, two members of the public jumped into the sea to rescue the child but failed due to rough sea currents. The coast guard staff finally rescued the child from the sea 3-5 minutes later; at the time, the child was unconscious. Resuscitation was performed for 3-5 minutes on the coast guard boat and continued for 45 minutes by ambulance staff. The child was pronounced dead at the scene. The family initially objected to a post mortem examination. It was then suggested to the corner and family that as the deceased was in the water for less than 1 hour and PMVSC would be useful in the diagnosis of drowning. PMVSC alone would offer 90% accuracy in the diagnosis of SWD, and an internal examination could potentially increase that certainty to >95%. If PMVSC did not suggest SWD, a full post mortem examination might be needed as other causes of death were possible. This was particularly important because the deceased may have had a natural event that caused him to collapse and fall into the sea, which could potentially have medical implications for the other twin. The family and coroner agreed to that approach, and a lesser post mortem examination was authorized.

12.2. Post mortem findings
A post mortem CT scan was performed prior to the post mortem examination and showed fluid in the airways and paranasal sinus, which is a non-specific sign of drowning. There were no injuries or natural disease processes identified on the body or on CT scan. No internal examination was performed.

12.3. Ancillary tests
Post mortem vitreous sodium chloride testing (PMVSC) was urgently requested, and the result was 286 mmol/L. Subsequent toxicology was negative for alcohol and drugs.

12.4. Cause of death
The cause of death in this case was SWD. The CCTV and witness statements provided an account of how and why the deceased fell into the sea. The rough current explained why the deceased was unable to remove himself from the water, as two other adults also had difficulty in the water. There were no injuries or natural disease found externally or on CT scan that could explain the death. Increased PMVSC confirmed a diagnosis of SWD without the need for a full post mortem examination.
12.5. Discussion

Traditionally, a suspected drowning death must undergo a full post mortem examination, but in cases with objection and strong contextual evidence, it would seem unreasonable. The presented case showed how the PMVSC can be applied in this type of case. This case points towards an accidental fall from a jetty resulting in SWD. It was reasonable for the family to object a post mortem examination, especially in a traumatic death involving a child. The police did not raise any suspicion or foul play regarding the death as there was CCTV footage and multiple witness statements. The coroner questioned what benefit was there for a full post mortem examination. It was advised to the coroner and family that analysing post mortem vitreous would benefit in this case to confirm SWD. The analysis of vitreous would be non-invasive, fast and have a high certainty. This would subsequently help in determining whether a full post mortem examination is necessary. After the authorization for a lesser post mortem examination, the PMVSC analysis took half an hour and showed an increase that was in keeping with SWD. This was relayed back to the coroner and family. In this case, the pathologist, police, coroner and family were all satisfied that the cause of death was SWD. The deceased was released back to the family without a full post mortem examination. This case illustrated how PMVSC can be very useful in giving a quantifiable diagnostic certainty and what potential improvement can be achieved if a full post mortem examination was performed when faced with a family objection. Although expressing an objection, the family would normally agree with a lesser post mortem examination without an internal examination, especially when a non-invasive procedure can provide a high diagnostic certainty. When advised, the family was very quick to agree to a lesser post mortem examination and understood that an increased PMVSC would support SWD, and a full post mortem examination would not be indicated. Conversely, if not increased, it would support a death not from drowning, and a full post mortem examination would be indicated.
Increased post mortem vitreous sodium and chloride levels and lung-body ratio provide high certainty in the diagnosis of salt water drowning

The case was a military-related death involving an individual who died during a military training exercise and was retrieved from salt water. This was a high profile death with major implications depending on the cause of death, such as occupational health issues, insurance compensation, and military training protocols and equipment review. A high level of certainty of the cause of death was required by the family, coroner, police and military.

In the presented case (case number: 17F0757), the post mortem was performed by me, and I have also given evidence regarding this case in military court.

12.6. Case history

A 42-year-old man had fallen from a height and was subsequently immersed in salt water during a military exercise. The deceased had no significant background medical history. On the day of his death, he and other members were participating in a training exercise that involved boarding a container ship from a rib vessel. During this exercise, the man climbed up a rope ladder that spanned from the rib boat to the container ship. At approximately 5-6 metres above the water surface, the rope became twisted, and he fell from the ladder and into the water. It was unclear whether he made contact with the rib boat or the container ship while entering the water. He was subsequently found lying face down in the water. He was subsequently found lying face down in the water. The other members involved in the training exercise were able to make contact with the deceased approximately one to one and a half minutes after immersion. By that time, the deceased was unresponsive, and his life jacket was not activated. He was airlifted back to port, and CPR and ventilation was continued; however, he passed away.

12.7. Post mortem findings

At the post mortem examination, the body was of an adult male with well-developed musculature. He weighed 77 kg (compared to his most recent medical check-up weight of 72 kg). There was a range of injuries on the body, with lacerations to the left side of the chin and the back of the head. These injuries did not result in any anatomically identifiable injuries to the skull and brain parenchyma. On the torso, there were bilateral rib fractures, bilateral
haemothorax, contusion to the right lung hilum, laceration of the pericardium, contusion to the heart, and hemopericardium. There were non-specific signs of drowning, which included froth in the airways, fluid in the stomach and waterlogged lungs (left-970 grams, right-1065 grams). The lung-body ratio was 26 g/kg. No macroscopic or microscopic natural disease processes were recognized at post mortem that would have caused or contributed to the death.

12.8. Ancillary tests

Toxicological analysis showed caffeine and trace levels of alcohol, probably from post mortem putrefaction. Biochemical analysis showed combined post mortem vitreous sodium and chloride levels of 300 mmol/L.

12.9. Cause of death

According to the tree diagram developed in chapter 11, the deceased had drowned in salt water, with a certainty of >95%. The cause of death was then formulated as a fall from height complicated by drowning. There was no medical explanation for why the deceased had initially fallen, and it was deemed to be accidental in nature. The injuries to the head may have been sustained during the fall, and an element of unconsciousness would be expected. The deceased was certainly alive while in the water and subsequently drowned. The chest injuries were probably secondary to chest compression from resuscitation and possible impact from the fall. The post mortem examination could not definitively separate which chest injuries resulted from the fall and which resulted from CPR.

12.10. Discussion

This case documented how post mortem vitreous sodium and chloride levels together with lung-body ratio can provide very high certainty in the diagnosis of SWD. This case had most of the classical findings seen in drowning deaths, although they were non-specific, and the deceased had a significant increase in body weight of 5 kg, most likely from salt water inhalation and ingestion. Due to the nature of the case, a very high diagnostic certainty was required by the interested parties. A mistake that determined that a natural disease process, such as a genetic cardiac condition, had precipitated the fall would have had major implications in terms of unnecessary medical investigations, downstream family follow-up and health screening processes for the military. The high diagnostic certainty of the diagnosis
of a fall complicated by drowning led to further military investigation into training and protocol and insurance compensation for the family.


Discussion

Chapter 13. Results and limitations

13.1. Summary of results

This thesis established post mortem vitreous electrolytes as a useful adjunct in the diagnosis of salt water drowning (SWD). The three sections examined different aspects of post mortem vitreous electrolytes in the diagnosis of SWD. The first section established that post mortem vitreous sodium and chloride levels (PMVSC, sum of vitreous sodium and chloride levels) are increased in SWD. During SWD, PMVSC initially increases as a result of drowning and subsequently increases from immersion. This property makes PMVSC a useful adjunct in the diagnosis of SWD when the immersion time is less than 1 hour. The second section established the use of post mortem vitreous magnesium level (PMVM) in the interpretation of PMVSC when the immersion time is greater than 1 hour or is unknown. PMVM increases as a result of salt water immersion, but not drowning. This differs from PMVSC, which increases initially as a result of drowning and subsequently from immersion. Given this difference, PMVM can be used to assess the effect of immersion when the immersion time is greater than 1 hour or is unknown. This finding allows the interpretation of PMVSC in these two situations. The third section compared the diagnostic certainty of PMVSC, lung weight (in the form of lung-body ratio, LB) and the combination of the two for the diagnosis of SWD. It demonstrated that the combination of PMVSC and LB offered the highest diagnostic certainty, followed by PMVSC and LB alone. A quantifiable improvement in diagnostic certainty was established when both PMVSC and LB were used in the diagnosis of SWD.

13.2. Practical applications

13.2.1. Diagnostic tool for salt water drowning

This thesis established post mortem vitreous electrolyte analysis as a useful adjunct in the diagnosis of SWD. Vitreous is the most proven and useful bodily fluid to sample and analyse at post mortem examination. It can be easily sampled with a needle and syringe and can be rapidly analysed in standard biochemistry analytical platforms, and its post mortem behaviour is well studied and established [43, 70, 73, 74, 107]. In terms of the diagnosis of SWD, post mortem vitreous electrolytes can provide reasonably high diagnostic certainty. Thus, post mortem vitreous electrolyte analysis is a useful adjunct in the diagnosis of SWD.
The initial studies of the use of vitreous as an adjunct to diagnosis a death by drowning 1970s and 1980s examined deceased individuals who had been immersed in water for more than 2 hours [54, 55, 75, 77]. With better understanding of the pathophysiology of drowning (namely, that death can occur within minutes), none of these studies examined drowning deaths with shorter immersion times. Chapters 2, 3, and 4 demonstrated that PMVSC increases in SWD, initially from drowning. During SWD, the inhalation and ingestion of salt water causes a rapid shift in plasma electrolyte levels, which then results in a significant increase in PMVSC. This initial increase in PMVSC as a result of SWD then plateaus for a period of at least 1 hour and subsequently increases due to the effects of diffusion and osmosis from immersion. Thus, PMVSC is a useful adjunct test in the diagnosis of SWD when the immersion time is less than 1 hour, and an increase in PMVSC supports the diagnosis. A PMVSC above 258 mmol/L has a diagnostic accuracy of approximately 90% (10% error rate), making it a useful adjunct test with high diagnostic certainty.

Chapters 6, 7 and 8 demonstrated that PMVM aids in the interpretation of PMVSC in deceased individuals who were immersed in salt water for more than 1 hour or for an unknown duration. When the immersion time is greater than 1 hour or is unknown, PMVSC cannot be interpreted because the effects of immersion may have caused PMVSC to increase. In SWD, magnesium is noted to be increased in the bodily fluids, indicating that magnesium can enter the circulatory system [86-90]. Interestingly, magnesium is relatively impermeable through the intact blood-brain barrier and can only pass through via active transport [93-95]. Clinically, the magnesium level in the cerebrospinal fluid remains constant despite the administration of intravenous magnesium or prolonged induced hypermagnesemia, and the transport of magnesium through the blood-brain barrier is mostly via slow active transport [91, 92]. Similar to the blood-brain barrier, the blood-ocular barrier prevents the free exchange of substances between the blood, retinal tissue and vitreous humour. Different from PMVSC, PMVM only increased as a result of salt water immersion and not drowning. Thus, when a deceased individual is immersed in salt water for less than 1 hour, PMVSC alone can be used as an adjunct in the diagnosis of SWD. When the immersion time is greater than 1 hour or is unknown, PMVSC should be interpreted with PMVM. If PMVM is not increased (normal: between 0.98-1.08 mmol/L), PMVSC can be interpreted because the effect of immersion on vitreous electrolytes would be minimal. If PMVM is increased, PMVSC cannot be interpreted because the effects of immersion will have set in.
13.2.2. Improved diagnostic certainty when vitreous electrolyte analysis is combined with lung-body ratio

Chapters 10 and 11 demonstrated that the diagnostic certainty of SWD can be further increased when both vitreous electrolyte levels and lung weight are interpreted. The LB ratio was shown to be a robust lung weight parameter with reasonable diagnostic certainty in the diagnosis of drowning (approximately 20% error rate), although it was not as accurate as PMVSC (approximately 10% error rate). However, when PMVSC and LB were used together, the diagnostic certainty improved (<5% error rate). In cases of death from SWD, both PMVSC and LB increase. However, there are instances other than SWD in which either PMVSC or LB can increase; for example, individuals who have underlying significant physical and mental conditions that result in low oral intake may show an increased PMVSC, but if they did not die from drowning, they may not present an increase in LB, while individuals who died from sudden cardiac deaths or drugs and alcohol- may have an increased LB with no increase in PMVSC. Thus, the combination of PMVSC and LB could more accurately separate deaths due to SWD (in which both PMVSC and LB would be increased) from non-drowning deaths (in which either or neither PMVSC and LB increases). This highlights the value of combining different predictors to improve diagnostic certainty.

13.3. Implication and applications

13.3.1. Suspected salt water drowning deaths

The traditional view in performing a full post mortem examination in all suspected SWD deaths is challenged in this thesis. Historically, a full post mortem examination is necessary in all suspected drowning deaths because there are no external or internal signs that are diagnostic of drowning [4, 5]. This thesis was able to establish post mortem vitreous electrolyte analysis as a useful adjunct in the diagnosis of SWD when the contextual evidence accords. PMVSC (together with PMVM) has a high diagnostic certainty, which is comparable to common clinical tests.

This finding has practical implications in the post mortem examination of suspected SWD deaths in New Zealand, especially when families have the right to object to a post mortem examination [81]. When there is an objection to a post mortem examination, a lesser post mortem examination is commonly authorized by the coroner and accepted by the family as a compromise to avoid a full post mortem examination that dissects the body. The lesser post
mortem examination allows vitreous to be collected and analysed. Post mortem vitreous analysis would be useful when the contextual information strongly points towards SWD, there is a strong family objection to a full post mortem examination and only a reasonable diagnostic certainty is required by the family, police and coroner. Post mortem vitreous electrolyte analysis and a lesser post mortem examination may provide sufficient medical evidence to make a diagnosis of SWD. Such an approach would be non-invasive and respectful of the family’s wishes while providing reasonably high diagnostic certainty of SWD within a short time frame. If the post mortem vitreous analysis does not support the diagnosis of SWD or cannot be interpreted due to the effects of immersion, the pathologist could advise the coroner, police and family that an alternative cause of death is probable and that a full post mortem examination is preferred. The family, coroner and police can use the information provided by the lesser post mortem examination to decide whether a full post mortem examination is necessary. A full post mortem examination could then be reserved for cases in which the contextual evidence is not strong, a high level of diagnostic certainty is required, and/or the post mortem vitreous analysis does not or cannot support a diagnosis of SWD. Thus, a full post mortem examination of all suspected SWD deaths would be unnecessary, and a lesser post mortem examination could offer a reasonable alternative in certain selected cases.

13.3.2. A stepwise diagnostic approach to the diagnosis of salt water drowning

The concept of a stepwise diagnostic approach to post mortem examination is applied in certain areas of Australia, but not in New Zealand. This practice is characterized by starting with a lesser post mortem examination and post mortem CT scan and proceeding to the dissection and examination of the body cavities one at a time until a full post mortem examination is completed. The pathologist, when satisfied that a reasonable cause of death can be established, can terminate the post mortem examination at any time. This method of post mortem examination is not validated or based on rigorous medical evidence.

This thesis demonstrated that an evidence-based stepwise approach to the diagnosis of SWD is feasible. In a case of suspected SWD death, post mortem vitreous electrolyte analysis performed in a lesser post mortem examination can provide a reasonably high diagnostic certainty for a diagnosis of drowning. A full post mortem examination can provide greater diagnostic certainty by assessing lung weight. The necessity of a full post mortem examination can be determined after the contextual information and lesser post mortem
examination results (with vitreous analysis) and the expected diagnostic certainty are considered by the pathologist, family, coroner and police. After the lesser post mortem examination with vitreous analysis, the pathologist can inform the interested parties that a diagnosis of SWD can (or cannot be made) with a diagnostic certainty of 90%, while proceeding with a full post mortem examination could increase diagnostic certainty to 95%. This would provide a quantitative stepwise evidence-based approach to the diagnosis of SWD. This stepwise approach would be ideal in cases in which the family, coroner, police and pathologist require information regarding what further diagnostic certainty can be gained from a full post mortem examination in the context of SWD and can make an informed decision about whether a full post mortem examination is necessary.

13.4. Limitations

13.4.1. Study design

The presented thesis has two major limitations in its study design, which were presented in the previous results sections. The first limitation is the use of enucleated bovine eyes to investigate the effects of salt water immersion on vitreous sodium, chloride and magnesium levels and the use of non-immersion deaths as controls. The second limitation is that the studies included in this thesis used salt water from local areas and may not represent the sodium and chloride levels of salt water in other parts of the world. These limitations are discussed in the previous sections, together with other minor limitations.

Another limitation is that this thesis did not take into account the effects of age and the presence of diabetes on post mortem vitreous electrolytes. Clinically, a transient time constant or lag exists between changes in blood and vitreous solute levels. Factors that can alter the rate of solute change in vitreous are blood-retinal barrier permeability and the vitreous diffusion coefficient. Diabetic retinopathy can impact vascular integrity, which increases blood-retina barrier permeability. The diffusion coefficient for solute movement in the vitreous is age dependent. With increasing age, the vitreous becomes more liquid and less gel-like, allowing solute to move more freely within the vitreous body. It is probable that the rate of solute change in the vitreous in response to changes in blood solute levels would be higher in an older individual with diabetic retinopathy than in a younger individual without diabetes. Hence, the age of the deceased and the presence of diabetes may impact post mortem vitreous electrolyte levels. Chapter 8 demonstrated that the PMVM level is
associated with diabetes (albeit with limited diagnostic ability) but not with age in adults. PMVSC has no documented relationship with age and/or diabetes in the literature [73], and this was not explored in our study. Although not controlled for, the effects of age and diabetes on PMVSC are expected to be insignificant compared to the effects of SWD and immersion.

13.4.2. Limitations as a diagnostic test

As discussed in chapter 1, drowning can be the final outcome of a number of initiating events, such as natural disease, trauma, and alcohol and/or drug toxicity. Post mortem vitreous electrolytes and lung weights (in terms of LB), as presented in this thesis, can only indicate that an element of SWD occurred. They provide no further information regarding the manner of death or any initiating factors. The tests and measurements used to aid the diagnosis of SWD are only adjuncts; they do not replace thorough contextual evidence gathering, and by no means do they replace the full post mortem examination, post mortem radiology and other ancillary tests.

Another major limitation is the interpretation of vitreous electrolytes when the effects of immersion have occurred. PMVSC is only useful to aid the diagnosis of SWD when the effects of immersion are absent. Thus, PMVSC can only be used when the deceased is immersed in salt water for less than 1 hour or when PMVM is not elevated. In our experience, a significant number of suspected immersion deaths are witnessed to be immersed in salt water for less than 1 hour and are retrieved quickly, making post mortem vitreous electrolyte analysis useful. However, when the immersion time is unknown or greater than 1-hour and PMVM is elevated, indicating that the effects of immersion have occurred, post mortem vitreous electrolyte analysis is of no use. Another potential parameter that is resistant to the effects of immersion and may have physiological and post mortem biochemical properties similar to those of the vitreous is cerebrospinal fluid. Its potential use to aid the diagnosis of drowning is discussed in the following chapter.
Chapter 14. Future directions

14.1. Drowning deaths

14.1.1. Combining other post mortem findings in salt water drowning

Post mortem CT scan findings and other internal post mortem findings could potentially be used to increase the diagnostic certainty of SWD in lesser and full post mortem examinations. The post mortem CT scan was introduced to Australia and New Zealand in the past two decades [126]. It has revolutionised the traditional post mortem examination, in which played an important adjunct role. Post mortem CT scans are part of the lesser post mortem examination and are widely accepted by pathologists, families, coroners and police. A post mortem CT scan can show signs suggestive of a drowning death, including fluid in the sphenoid sinus and airways, changes in lung marking patterns and opacity, and increased stomach content density [127, 128]. These findings, together with post mortem vitreous analysis, would add further diagnostic certainty when a lesser post mortem examination is performed using analytical techniques similar to those presented in chapter 11. Other traditional macroscopic and microscopic morphology findings, such as the “plume of froth”, pleural effusion, presence of fluid in the stomach and emphysema aquosum on histology, may be incorporated, yielding an even higher diagnostic certainty.

14.1.2. Use of cerebrospinal fluid electrolytes

A major limitation in the use of post mortem vitreous in SWD is the effects of immersion. When the immersion time is greater than 1 hour or is unknown, and PMVM is increased, PMVSC would be uninterpretable due to the effects of immersion. This would void the use of post mortem vitreous biochemistry as an adjunct in the diagnosis of SWD. Recently, cerebrospinal fluid (CSF) was suggested as a promising alternative to aid in the diagnosis of drowning in animal models [129]. It was observed that in rabbits, CSF sodium and chloride levels increase in SWD. CSF, albeit more difficult to sample, analyse and interpret than vitreous, is less prone to the effect of immersion which gives it an advantage over vitreous.

If CSF is proven to be useful in suspected human SWD death, it would be incorporated with vitreous analysis. In suspected SWD, vitreous would be the first sample to be analysed due to its ease of sampling. If the effects of immersion had occurred based on an increased PMVM, CSF biochemistry would be another adjunct biochemical test for use in diagnosing SWD.
However, post mortem CSF sampling, analysis and interpretation are much more complex and less studied. Post mortem CSF can be sampled via lumbar puncture, aspirating the subarachnoid cisterns around the neck or aspirating the cerebral ventricles, and the biochemical differences between these sites at post mortem have been documented (original article published in American Journal of Forensic Medicine and Pathology. Garland J, Philcox W, Kesha K, Morrow P, Lam L, Spark A, Palmiere C, Elstub H, Cala AD, Stables S, Tse R. Differences in Sampling Site on Postmortem Cerebrospinal Fluid Biochemistry: A Preliminary Study. Am J Forensic Med Pathol. 2018 Dec;39(4):304-308) [130]. The reference value of CSF is not as well established as that of vitreous [130]. Furthermore, a change in post mortem CSF biochemistry in human SWD deaths is only reported in one case report (accepted case report published in American Journal of Forensic Medicine and Pathology. Garland J, Philcox W, Kesha K, McCarthy S, Lam L, Palmiere C, Hensby-Bennett S, Stables S, Tse R. Elevated cerebrospinal fluid sodium and chloride levels in salt water drowning death. Am J Forensic Med Pathol. 2019 (in-press)). Further data collection and planned studies are underway to better understand the post mortem characteristics of CSF biochemistry and its application to drowning death.

14.1.3. Fresh water drowning

This thesis examined SWD deaths as they are encountered more frequently than fresh water drownings at the centres where the presented studies were carried out. Fresh water drowning deaths in rivers, lakes, swimming pools, or baths are not infrequently encountered in forensic pathology [4, 5], and we collected a handful of definite fresh water drowning deaths that occurred in fast-moving rivers, bathtubs and swimming pools. Of interest, we also collected a cluster of fresh water drowning deaths during a flash flood incident. All these cases showed a recorded decrease in PMVSC. This observation was the opposite of the PMVSC findings for SWD and was in keeping with the working hypothesis of this thesis. Further studies using similar methods are needed to establish the cut-off for PMVSC in fresh water drowning deaths. An extension that combines post mortem CT, CSF and traditional post mortem findings, as discussed above, would also be expected.
14.2. Non-drowning deaths

14.2.1. Application of the statistical learning model

The analytical method presented in chapter 11 is new to forensic pathology practice in New Zealand and can be applied to non-drowning deaths. The analytical method used was based on basic statistical/machine learning techniques, which form the foundations of artificial intelligence and deep learning [131]. This type of analysis is more widely used in non-medical disciplines and has been adopted more slowly by health care, clinical medicine and pathology [131, 132]. This analytical method is designed to predict an outcome based on an array of predictors; in this thesis, the method was designed to predict SWD using post mortem vitreous electrolyte levels and lung weights. The concept of predicting a categorical or a continuous outcome with a combination of categorical or continuous predictors could be useful in the field of medical diagnosis. This is not a foreign medical concept, as the idea of using the combination of clinical history, clinical signs, imaging results and pathological tests to make a diagnosis is applied by doctors daily. As few clinical findings are perfect in terms of specificity and sensitivity, the interpretation of a single clinical finding to diagnose a particular condition would, not surprisingly, be inaccurate. However, interpreting multiple clinical findings in combination could lead to an increase in diagnostic certainty. This concept can be applied similarly to forensic pathology using the post mortem equivalent of clinical history, clinical signs, imaging results and pathological tests (i.e., contextual evidence, post mortem examination, post mortem CT scans and ancillary tests). Thus, the analytical approach used for SWD deaths presented in this thesis could be extended to include more predictors (i.e., contextual evidence, post mortem examination, post mortem CT scan and ancillary test) and applied to non-drowning deaths.

14.2.2. Reduction of the full post mortem examination rate

The results of this thesis and the illustrative case reports provide an evidence-based stepwise approach to allow the coroner and family to make an informed decision regarding how best to proceed with a post mortem examination in the context of SWD. In the illustrative case reports presented in chapter 12, the pathologists were able to use a lesser post mortem examination and post mortem vitreous analysis to inform the family and coroner of the diagnostic certainties of the two presented cases. Depending on the degree of diagnostic certainty required and the post mortem vitreous electrolyte results, a full post mortem
examination was either avoided or pursued. This approach, compared to the traditional approach in which a full post mortem examination is always required, could potentially reduce the rate of full post mortem examinations in suspected SWD deaths.

This stepwise approach, presented in the context of SWD, could potentially form a framework for reducing the rate of full post mortem examinations in non-drowning death cases. Presently, in New Zealand, there is a push towards lowering the rate of full post mortem examinations to reduce the costs to the government and the risks to the family while maintaining high diagnostic certainty regarding the cause of death. It is agreed that a full post mortem examination is indicated for certain types of deaths, such as suspicious deaths and homicides. However, there is a group of cases for which a full post mortem examination would not add further value in establishing a cause of death. Examples include suicide deaths by hanging, deaths in which a definite cause of death is established by post mortem CT scans, and certain traumatic cases [133-135]. These cases may not require a full post mortem examination if a lesser post mortem examination with post mortem CT scans can determine the cause of death and address any concerns raised by family, police and coroners. There is, however, a large group of cases in which it is unclear whether a full post mortem examination would add further information to establish a cause of death. A method for extracting and analysing the findings from the lesser post mortem examination (e.g., contextual evidence, external examination results, post mortem CT scan findings and ancillary test results), estimating the diagnostic certainty of a particular cause of death and subsequently determining what a full most mortem examination could add to the diagnostic would certainty help the family, coroner and pathologist to decide whether a full post mortem examination is required. This approach could potentially reduce the overall full post mortem rate in an evidence-based manner.

14.2.3. Diagnostic certainty

This thesis used statistical analytical techniques to ascribe diagnostic certainty in a SWD death using post mortem vitreous levels and lung weights, a technique that is novel to forensic pathology practice in New Zealand. The presented study methodology and analytical technique can be similarly applied to non-drowning deaths.

Deaths encountered in forensic pathology commonly have multiple competing possible causes of death and overlapping post mortem signs. A common issue forensic pathologists face is the need to determine the diagnostic certainty of a particular cause of death for the
family, police, coroner, and/or court. The findings used to determine the cause of death during the post mortem examination include contextual evidence, post mortem CT scans, the post mortem examination itself and post mortem ancillary tests. Pathology findings that are not compatible with life, such as the acute rupture or occlusion of major blood vessels or non-survivable trauma, are straightforward but represent only a small proportion of the deaths encountered. Large proportion of cases have multiple non-specific pathological findings and thus may have a range of competing causes of death, but a quantifiable diagnostic certainty is seldom provided. Additionally, in some instances, a cause of death can be sequential or in combination. For example, death can be caused by an unnatural event that exacerbated a natural condition (such as death following physical exertion in a person with established heart disease), a natural condition that precipitated an unnatural event (such as multiple injuries sustained from a fall after an epileptic seizure) or multiple natural and/or unnatural events acting in combination and leading to death. Thus, determining the cause of death can be very difficult, with layers of factors acting in sequence or in combination. Having a robust database, building more complex predictive models that can analyse a range of data and addressing the complexity of ascribing a cause of death and determining the diagnostic certainty are possible future research directions in both drowning and non-drowning deaths.

Furthermore, another potential research area is the determination of diagnostic certainty of different post mortem findings. A ceiling effect is anticipated when more post mortem findings are collected and analysed with no further improvement in the diagnostic certainty. To analyse which post mortem finding, or group of findings, is the most discriminatory can help stratify what test should be performed and what post mortem finding should be documented. This would enable pathologists to be more efficient in their post mortem examination.
Conclusion

This thesis demonstrated that post mortem vitreous electrolytes are a useful adjunct in the diagnosis of salt water drowning (SWD). It has a high diagnostic certainty, and when combined with lung-body ratio (LB), a further increase in diagnostic certainty was demonstrated. The implication of these results and the analytical method used has important implications for diagnosing drowning and non-drowning deaths.

The traditional approach to suspected SWD death, in which a full post mortem examination is necessary in all cases to establish a cause of death, was challenged. When the contextual evidence accords, high diagnostic certainty is not required, and the family objects to a full post mortem examination, post mortem vitreous electrolytes can be a very useful aid in the diagnosis of SWD. Increased post mortem vitreous sodium and chloride levels (PMVSC) when the deceased individual was a) immersed in water for less than 1 hour or b) immersed in water for more than 1 hour or for an unknown time and post mortem vitreous magnesium (PMVM) is normal would support the diagnosis of SWD. Such findings would avoid the need to perform a full post mortem examination in all suspected drowning deaths. When the contextual evidence is not strong, high diagnostic certainty is required, and/or PMVSC is not elevated or is uninterpretable due to the effects of immersion (when the immersion time was greater than 1 hour or unknown and PMVM is increased), a full post mortem examination would be indicated.

The combination of post mortem vitreous analysis (PMVSC and PMVM) and LB (determined by a full post mortem examination) would substantially increase the diagnostic certainty of SWD. The analytic method used to determine the diagnostic certainty of SWD based on post mortem vitreous, LB, and the combination of both represents a novel approach to suspected SWD. This analytical method used statistical/machine learning techniques, the foundations of artificial intelligence, to provide and compare the diagnostic certainty of different pathology findings separately and in combination. These techniques are more established in non-medical disciplines and other branches of medicine but are new to forensic pathology practice in New Zealand. They are powerful techniques that can be applied to forensic pathology to establish a cause of death and assess the value added at each step of the post mortem examination. With an evidence-based approach, such techniques could have significant implications for the quantification of diagnostic certainty and the reduction of full post mortem examination rates.
Future research into drowning deaths and non-drowning deaths is anticipated. Post mortem CT scans, cerebrospinal fluid biochemistry and traditional anatomical post mortem findings can be combined to increase the diagnostic certainty of SWD in both lesser and full post mortem examinations. The study method and analytical technique can be extended to fresh water drowning deaths and mirrored for non-drowning deaths. Major challenges include data collection and analysis, which require a robust database to handle and analyse the complexity of determining a cause of death. The aforementioned potential research areas and challenges are being addressed in New Zealand. A national database system for data management and analysis is being created, which will make further research more accessible in the future.
References

59. Garland, J., et al., *Elevation of post mortem vitreous humour sodium and chloride levels can be used as a reliable test in cases of suspected salt water drowning when the immersion times are less than one hour*. Forensic Sci Int, 2016. 266: p. 338-342.


