U.PORTO

MASTER IN LEGAL MEDICINE

Variability in response to traumatic injury considering genetics and pathophysiology of the disease

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2025

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Variability in response to traumatic injury considering genetics and pathophysiology of the disease

Dissertation submitted in parcial fulfilment of the requirements for Marter's Degree of Legal Medicine to Insituto de Ciências Biomédicas de Abel Salazar da Universidade do Porto

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Acknowledgements

Completing this dissertation marks the end of a demanding yet enriching journey, filled with challenges, learning, and personal growth. None of this would have been possible without the support of several people, to whom I extend my deepest gratitude.

To my parents, thank you for your unconditional love, constant support, and for being a source of strength and inspiration throughout my life. Your presence has been fundamental every step of the way.

To my sister, for always being there, for your encouragement, and for reminding me to believe in myself, .

To my boyfriend, for your unwavering support, patience, and understanding, and for walking beside me through each stage of this journey. Your presence truly made a difference.

To my friends, thank you for your companionship, for your kind words during difficult times, and for celebrating every milestone with me. Your support helped keep me motivated.

To my mentor, I am truly grateful for your academic guidance, insightful feedback, and continuous support throughout this process. Thank you for your availability, your trust in my work, and for inspiring me to grow as a researcher.

To the programme coordinators and all my lecturers, thank you for the valuable knowledge you have shared, for your dedication to teaching, and for your important role in shaping my academic path.

To my fellow students, first line in class, thank you for the shared experiences, the mutual support, and for the camaraderie that made this journey lighter.

To all of you, my heartfelt thanks.

Abstract

Genetics have an influence on the response to injuries. Furthermore, traumatic injuries are heterogeneous, and their response is individual and varies due to genetic and pathophysiological factors.

This dissertation aims to investigate various traumatic injuries and the different responses to these injuries. The genetic variability of each individual has been taken into account, as well as the physiology of the disease that can influence the outcome of the traumatic injury, potentially leading to death.

This theoretical and literature review study aims to demonstrate the correlation of biology and genetics with death, in this case, traumatic death, associating my academic background – biology degree and master's degree in legal medicine.

Finally, the results of this project contribute to this growing field and that are important for confirming assumptions, to improve therapeutic interventions and to a better general understanding of this theme.

Keywords: genetics, traumatic injury, pathophysiology, response to injury, legal medicine.

Resumo

A genética tem influência na resposta à lesão. Mais especificamente, as lesões traumáticas são heterogéneas e a resposta às mesmas é individual e varia devido a fatores genéticos e fisiopatológicos.

Esta dissertação tem como objetivo principal investigar empiricamente as lesões traumáticas e a influência da variabilidade de resposta às mesmas, tendo em conta a variabilidade genética de cada indivíduo e também a fisiopatologia de doença, podendo levar ou não os indivíduos à morte.

Este estudo, teórico e baseado em revisão de literatura, pretende demonstrar a relação da biologia e genética com a morte, neste caso, morte por lesões traumáticas, relacionando assim, as áreas da minha formação: da licenciatura – biologia - e do mestrado – medicina legal, respetivamente.

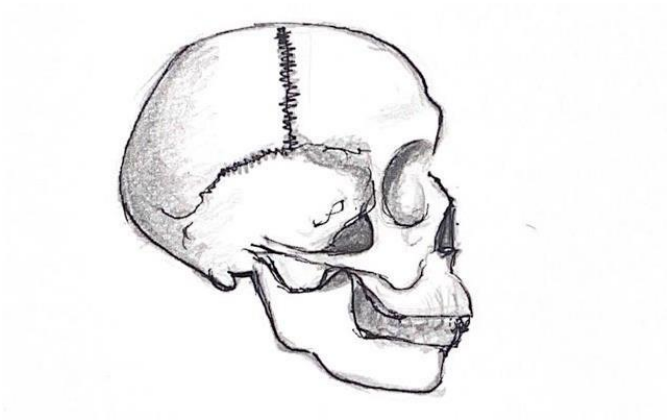
Além disso, este projeto contribui para o crescimento de um tema da medicina legal que não é muito estudado e os resultados encontrados serão importantes para a confirmação de premissas, melhoria de intervenções terapêuticas e para uma melhor compreensão geral deste tema.

Palavras-chave: genética, lesões traumáticas, fisiopatologia, resposta à lesão, medicina-legal

“O CADÁVER É UM TRATADO.

O CADÁVER FALA, O QUE É PRECISO É SABER FALAR COM ELE.”

- Prof. Dr. J. Pinto da Costa



Indice

Figure Indice

1. Introduction

Trauma is one of the most common causes of death all over the world. Even though, there is not enough statistical data, since this issue has not been sufficiently investigated, specifically in Europe.[17] Although the USA is the country that presents more numbers in this matter, this study will be focused in Europe, as long as the research makes it possible to. In Portugal, there are very few studies regarding this topic.

The concept of trauma is very broad and heterogeneous, due to its causes, types of injuries, severity and individual genetic variations. Therefore, there is a wide rage of trauma, as well as in associated individual response and in mortality rates associated with it.[14] Traumatic brain injury -TBI- is one of the most fatal forms of trauma, responsible for a large proportion of deaths, for this reason is one of the most studied forms of trauma. The complexity of TBI is further exacerbated by the heterogeneity in patient outcomes, even among similar injuries, ages and health statuses. [4]

The study of genetics in trauma response has advanced significantly in recent decades, providing deeper insights into the factors that influence the outcome of traumatic injuries. Interindividual variation, genetic and pathophysiological factors play a crucial role in modulating inflammatory responses, coagulation processes, energy metabolism and biological processes that can influence the outcomes of trauma victims. Animal model studies have demonstrated the significant role of genetic background in determining recovery extent after injur. [4] While human studies have identified candidate genes influencing recovery. However, this field remains in early stages, often relying on candidate genes studies with limited patient numbers. [6]

Integrating genetics and pathophysiology into the study of traumatic injuries not only helps understand interindividual variability in trauma response but can also hold great relevance for forensic medicine, assisting in determining the cause of death and explaining unexpected fatalities in trauma victims. In legal cases, such information can be crucial in clarifying whether an individual had a genetic predisposition to an unfavorable trauma response or whether the fatal outcome was solely due to the severity of the injury.

The aim of this study is to critically review and analyze scientific literature on the influence of genetic factors on the response of fatal traumatic injuries. To do so articles that contain information on key biomarkers, genetic polymorphisms, as well as pathophysiological mechanisms involved in trauma response in the light of the forensic field were reviewed.

A comprehensive understanding of this matter will potentially contribute to the development of more effective investigations and develop this forensic field.

2. Methods

A comprehensive search of relevant and reliable databases, including Google Scholar and PubMed, was conducted using keywords such as “traumatic injury”, “traumatic deaths”, “genetics”, “response to injury”, “legal medicine”, “forensics”, “pathophysiology”. The vast majority of the selected articles were published in English. Initial screening was based on abstracts and conclusions, focusing on studies that explicitly examine the association between genetic variations and response to trauma. Subsequently, full text articles were evaluated regarding the methodologies used and their relevance to the research questions. More studies focused on traumatic brain injury (TBI) were conducted than those that examined general trauma. Consequently, the influence of genetic factors on TBI was also more frequently investigated.
The data extraction aimed to summarize the main findings, including specific genes, types of traumatic injuries studied, and the outcome measures used. A critical evaluation of each study’s limitations was also conducted, such as sample size and potential confunsing factors. The synthesis of the extracted data focused on identifying consistent patterns and discrepancies among studies. By synthesizing the existing literature, this review aims to provide a comprehensive overview of the current understanding of the genetic basis of variable responses to traumatic injury.

3. Literature review

3.1. Traumatic injuries

In forensic pathology, a wound or injury is defined as structural compromise to bodily tissues resulting from the application of external mechanical forces.
Trauma refers to any injury to living tissue caused by an extrinsic agent. The classification of traumatic injuries is based on the mechanism of injury and the appearance of the wound.
The human organism is continuously exposed to a spectrum of mechanical stressors, ranging from constant gravitational forces to acute impacts during different physical activities.

Tissue damage occurs when the magnitude of the applied force surpasses the resistive capacity of the affected tissues. The intensity of the applied force is governed by principles of classical mechanics, demonstrating a direct proportionality to the mass of the impact object or weapon and the square of its impact velocity. The weapon can encompass a wide array of objects, including the human body itself, as frequently observed in scenarios involving rapid deceleration, such as falls or vehicle collisions. Additionally, the spatial distribution of the force, specially the area over which it is applied, is a critical determinant of tissue damage. (16)

The effects of excessive mechanical forces on biological tissues manifest as compression, traction, torsion, tangential (shear) and leverage stresses. The resultant tissue damage is contingent upon both the nature of the mechanical insult and the intrinsic properties of the target tissue.

Mechanisms that can produce injuries are classified as blunt force mechanisms, sharp force mechanisms, perforating mechanisms and combined injury mechanisms.

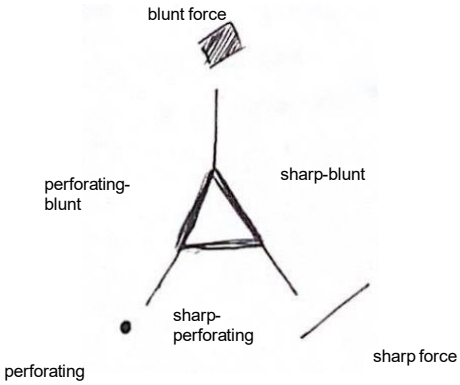


Figure 1 - Injury mechanisms

Given the prevalent involvement of the skin in traumatic injuries, it is justified to make a concicise review of the architecture of the skin and subcutaneous tissue, along with its associates terminology. This is particularly relevant in a forensic context, as it allows for an assessment of the force requires to breach the cutaneous barrier and induce bleeding from the underlying vascular structures.

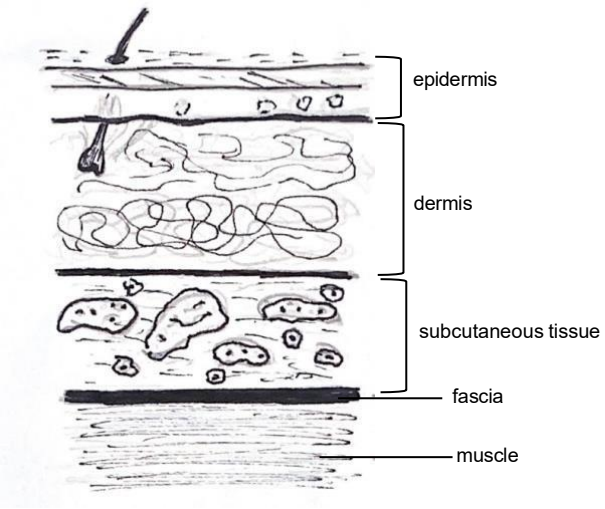


Figure 2 - Skin

The epidermis, the outermost layer of the skin, comprises living cellular strata in conjunction with the stratum corneum, a non-vascularized, keratinized layer. The dermo-epidermal junction exhibits a corrugated morphology, characterized by epidermal ridges (papillae) that interdigitate with the underlying dermis. The degree of this undulation varies topographically, with regions of thinner skin typically displaying a less pronounced interdigitation.

The dermis, also known as the corium, is composed of a complex matrix of connective tissue that houses various skin appendages, including hair follicles, sudoriferous glands and sebaceous glands. It is richly vascularized and innervated, containing a dense network of blood vessels, lymphatic vessels and nerve fibers. Specialized sensory receptors within the dermis mediate tactile perception, pressure sensitivity and thermal sensation. The deeper aspect of the dermis, the subcutaneous tissue, is characterized by the presence of adipose tissue. Depending on anatomical location, deep fascia, adipose tissue and underlying musculature will from distant strata beneath the cutaneous layers. [16]

The application of a blunt force, delivered through a rigid object with a flat surface, results in considerable mechanical energy on the skin. Typically, such impacts can induce trauma to the superficial epidermal layers (abrasion) or they can reach the dermis, causing rupture of blood vessels (bruise) in both cases without disruption of tissue continuity or loss of substance. However, in higher-magnitude forces, the structural integrity of the cutaneous barrier may be compromised, leading to discontinuity and extending the zone of injury to deeper underlying tissues (laceration).

Sharp force trauma is characterized by a cutting mechanism involving the application of pressure combined with sliding motion of a bladed instrument across the body surface, resulting in a disruption of the skin's structural integrity. The depth of tissue penetration varies depending on the applied force and the sharpness of the instrument, affecting different levels of the cutaneous and subcutaneous tissues. Incised wounds are defined by exhibiting smooth, regular and well-defined margins, where the length exceeds their depth. These wounds typically display an initial and terminal tail, lack tissue bridging within the wound path, present angular extremities and generally without associated contusion artefacts.

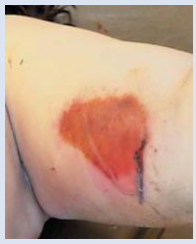
Figure 3 - Sharp force injury

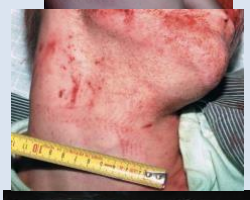
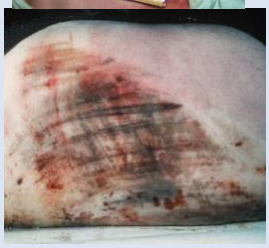
Perforating trauma arises from the application of a substantial force concentrated on a reduced surface area, resulting in a wound tract that is deeper than its surface dimension. The resultant injury involves the disruption of the skin's integrity and of multiple structures along the weapon path, with the extent and nature of the damage contingent upon factors such as trajectory, orientation, magnitude of the applied force along with the characteristics and size of the penetrating object or weapon are also very important.

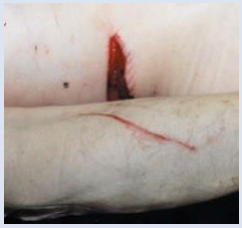
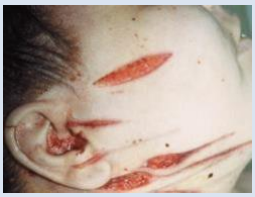
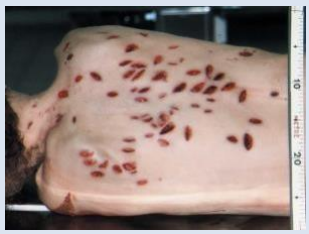
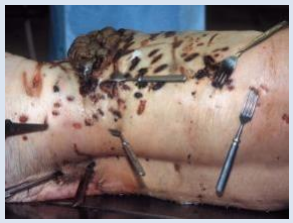
Combined injury mechanisms encompass a spectrum of traumatic injuries, including sharp-penetrating, sharp-blunt and perforating-blunt mechanisms (injuries resulting from firearm projectiles).

Table 1 - Types of injuries; Figure 4-13 – Images of injuries. (16)

Mechanisms	*BLUNT FORCE*	*SHARP FORCE*	*PERFORATING FORCE*
Types of injuries	Abrasions, patterned abrasions bruises, lacerations	Incised wounds	Perforating wounds



Other types of traumatic mechanisms that may occur, although less frequently, include asphyxia, thermal trauma, chemical trauma and electrical trauma.

The fundamental purpose of respiration is the transfer of atmospheric oxygen to the body's peripheral tissue cells. Any impediment to this oxygen transfer can be broadly termed 'asphyxia', although the more precise terms 'hypoxia' (a reduction in oxygen supply) or 'anoxia' (a complete absence of oxygen) are preferable for diagnostic accuracy.

Examples of such impediments include:

1. Obstruction of external respiratory orifices, such as through smothering or gagging.
2. Blockage of internal respiratory passages at pharyngeal, laryngeal, tracheal, or bronchial levels.
3. Restriction of thoracic respiratory movements, preventing the inspiration of air despite patent airways, a phenomenon observed in 'traumatic asphyxia'.

A significant challenge for pathologists is the difficulty in reliably equating specific autopsy findings of 'asphyxia' with measurable physiological hypoxia. (16)

Suffocation is typically defined as death resulting from a reduction in the oxygen concentration of the respired atmosphere. More commonly, oxygen reduction occurs through the physical displacement of air by other gases or via chemical changes such as those in combustion. In fires, the presence of toxic gases like carbon monoxide and carbon dioxide is typical. However, devices that burn kerosene or natural gas can cause death purely from hypoxia, particularly if operated overnight in a confined, small room with sleeping occupants. (16)

Smothering refers to death caused by the mechanical occlusion of the mouth and nose, often by fabric. Death in these instances may result from the occluding material pressing against the facial orifices or from the passive weight of the head creating an airtight seal. Smothering can also occur when a pad or gag is secured over the face - gagging as may happen during violent robberies. (16)

Choking involves the blockage of internal airways, typically between the pharynx and the tracheal bifurcation. While death can result from pure hypoxia due to airway occlusion, a substantial proportion of fatalities occur abruptly, preceding any demonstrable hypoxic manifestations. These sudden deaths are attributed to neurogenic cardiac arrest, potentially exacerbated by excess catecholamine release during the adrenaline response. Causes of choking include foreign objects, dentures, haemorrhage or food material. (16)

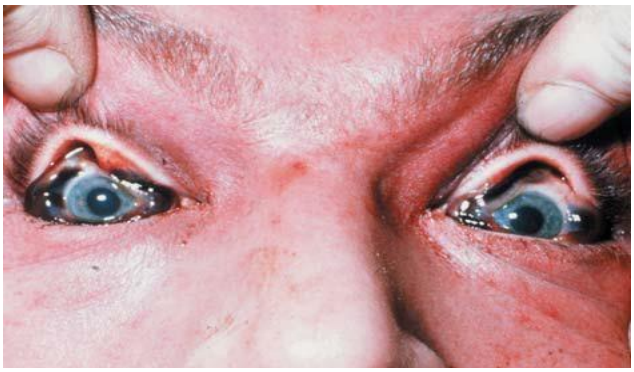


Figure 5 - Gross conjunctival hemorrhage in traumatic asphyxia (16)

'Traumatic asphyxia' impedes respiration by restricting thoracic movements, thereby preventing inspiration. This condition is termed 'traumatic' due to the gross mechanical forces that typically cause thoracic cage fixation. Traumatic asphyxia commonly presents in two scenarios: firstly, where the chest and often the abdomen are compressed by an unyielding object or substance, such as during burial, entrapment under an overturned vehicle, or crushing by falling timber or masonry, preventing chest expansion and diaphragmatic descent. Secondly, it can occur due to crushing within crowds or mass disasters. Individual cases may arise when one person exerts their body weight on another for a prolonged period. [16]

Regarding thermal injuries, the extent of damage from applied heat is contingent upon the temperature; the body's capacity to conduct away excess heat and the duration of exposure. It is crucial to recognize that even relatively low temperatures, around 44°C, can inflict damage if sustained over time. Radiant heat, such as from excessive sun exposure or artificial sunlamps, can also cause severe injury. Burns are categorized into first, second, and third degrees, with increasing severity and consequences for the body, potentially leading to death. A 'scald' denotes tissue damage caused by hot liquids, most commonly water, but also oils, molten materials, and steam. Unlike dry heat burns, scalds typically do not result in charring, carbonization, or singeing of hair, unless caused by superheated oils. When a hot liquid is thrown or splashed, makes a figured injury in the body, leaving a specific autopsy finding. Burns from dry heat result from conduction or radiation and, similar to scalds, the degree of tissue damage is dependent on temperature and duration.

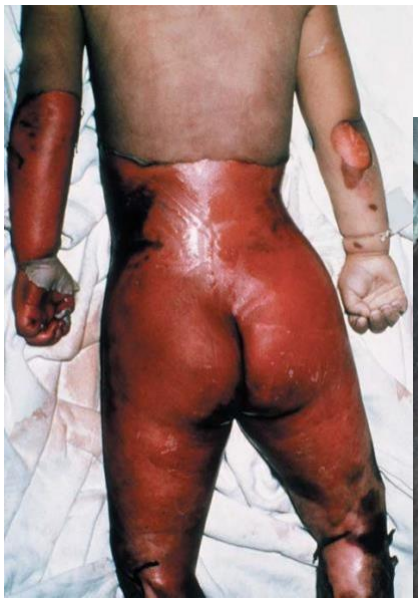


Figure 6 - Scald - heat burns (16)

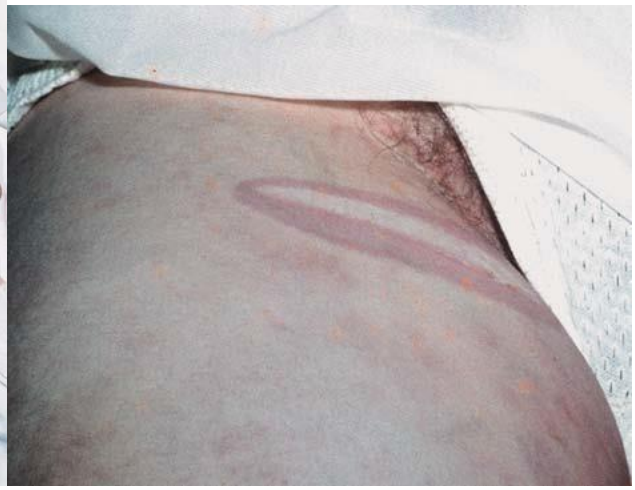


Figure 7 - Dry heat burn (16)

In case of hypothermia, the body is in a state of abnormally low body temperature, that can be precipitated by ambient air temperatures below approximately 10°C, particularly when exacerbated by air movement such as wind or internal draughts, which significantly increase the rate of bodily heat loss. As core body temperature declines further, individuals may experience dulling of consciousness, reduced respiratory and heart rates, and a drop in blood pressure. If prolonged without intervention, this condition can lead to death, with recovery becoming increasingly unlikely at these temperatures. While the elderly and the very young are recognized as being at the highest risk, fatal hypothermia can affect healthy adults, infants, and the elderly alike. This can occur due to environmental conditions that are not necessarily extreme. A common scenario, particularly in temperate and colder climates, involves intoxicated individuals exposed to even moderate cold. Typical circumstances include an inebriated person who falls asleep outdoors or sustains an incapacitating accident, such as a fall or minor head injury, leading to prolonged immobility and subsequent hypothermia. Alcohol appears to play a dual role: firstly, by inducing incapacitation and immobility, and secondly, through its vasodilatory effect on the skin, which accelerates heat loss. At higher concentrations, alcohol may also exert a direct inhibitory effect on the body's thermoregulatory centres. Naturally, the risks are amplified in colder geographical regions and at high altitudes. (16)

The spectrum of substances capable of inducing fatal poisoning is extensive, encompassing a wide array of chemical agents.

It is crucial to recognise that the concept of a fixed "fatal dose" is biologically imprecise, given the significant inter-individual variation in responses influenced by genetic factors. Consequently, the accurate investigation of suspected poisoning fatalities hinges critically on meticulous sampling of biological fluids and tissues from the deceased. Drugs of dependence represent a primary category of chemical agents causing fatalities, often through routes of administration including oral ingestion, intravenous, subcutaneous, or intramuscular injection, inhalation (smoking), or nasal insufflation. Prominent examples include heroin, cocaine, methadone, hallucinogens, cannabis, and amphetamines. The ongoing advancement in pharmaceutical development continually introduces novel, often more hazardous, substances. Autopsy findings in deaths attributable to these drugs are generally non-specific, necessitating comprehensive toxicological analysis and expert interpretation for accurate elucidation of the cause of death. [16]

Alcohol-related fatalities can arise from the direct central nervous system depressant effects on the brainstem, particularly the respiratory centres, or secondary complications such as the aspiration of gastric contents. Alcohol intoxication is frequently implicated in various forms of fatal trauma, including road traffic accidents involving either intoxicated drivers or pedestrians, and is a common precipitating factor in falls. In developed economies with advanced medical services, poisoning by medicinal compounds is prevalent, frequently exceeding fatalities from other toxic agents, particularly in cases of suicide and accidental ingestion. Commonly implicated therapeutic agents include analgesics (such as paracetamol), antidepressants, benzodiazepines, and phenothiazines. The concentration required to cause death is substance-specific, influenced by its physiological effects and the individual's genetic makeup. Following alcohol and certain pharmaceuticals, carbon monoxide poisoning is one of the most frequently encountered toxicological emergencies. It is generated during the incomplete combustion of fossil fuels, and its high affinity for haemoglobin means that even low concentrations can lead to cumulative toxicity. The potent chemicals utilized in agriculture pose risks of accidental exposure during application or due to improper storage, and are also a significant route for self-administered poisonings, particularly for suicide. The primary hazards in agricultural chemicals lie with pesticides, notably organophosphorus compounds and herbicides. Toxicity can occur via ingestion, inhalation, or dermal absorption, with lethal potential dependent on the specific compound and concentration.

These agents can induce tissue necrosis, oedema, haemorrhages, and muscular hyperexcitability, with the lungs, gastrointestinal tract, and liver being the principal organs affected. Corrosive poisonings, while less common than historically, still contribute to fatal outcomes. The damage inflicted by corrosive substances is primarily structural rather than systemic toxicity, unless complications such as renal failure or secondary chest infections arise in survivors. Common features include characteristic corrosive patterns on the skin, erosion of the oral mucosa (with the tongue potentially being swollen or shrivelled depending on the agent), and damage to the respiratory tract from aspiration of liquids or vapours, leading to rapid pulmonary oedema and haemorrhages. The lower oesophagus and stomach are also susceptible to damage, including discoloration, desquamation, and potential perforation. Fatalities can result from pulmonary oedema due to lung aspiration; if survival extends beyond a day, fulminant bronchopneumonia may be the terminal event. [16]

Electrocution necessitates a pathway for electrical current across the body, crucially involving vital structures in fatal cases. The current typically enters at one point, frequently a hand gripping an electrical device, and exits elsewhere, usually to an earth or neutral conductor. Fatal electrocution can happen in three primary life-threatening events: most commonly, current passage across the heart causes fatal cardiac dysrhythmia, typically ventricular fibrillation leading to asystole. Less frequently, current passing across the chest and abdomen can induce respiratory paralysis via spasm of the intercostal muscles and diaphragm. Rarely, current passing through the head and neck may directly paralyze the cardiac or respiratory centres in the brainstem. The presence or absence of a visible lesion following current passage depends on the current density relative to the skin area and its conductivity, which is often influenced by moisture content. [16]

3.2. Most significant traumatic injuries

Injuries in the head and neck, usually emerging from blunt force trauma, are the most prevalent and significant.

This importance can be attributed to several factors:

- the head is frequently the primary target in most blunt trauma assaults;
- impact from falls, as victims often sustain injuries when pushed or knocked to the ground, as they typically strike their head upon impact;

- the vulnerability of the brain as the brain itself and its protective coverings are susceptible to blunt trauma.

The above-mentioned mechanisms may not be fatal if inflicted on other body areas. (1)

Head injuries provide the major contribution to death in assaults, falls and transportations deaths. Fatalities can occur even from falls from a standing position, while some individuals may survive to falls from significant heights. Head injuries, particularly those resulting from falls onto the back of the head, can be lethal. When a person falls, the body may maintain its orientation to the ground, but it often twists and turns unpredictably. The extent of this alteration in posture is influenced by the height of the fall and the time available for the body to adjust.

Fall directly onto the head can lead to severe fractures, scalp lacerations and potential brain extrusion. Conversely, falling onto the side of the body can result in a variety of injuries including multiple rib fractures, shoulder or arm fractures and severe abdominal injuries, which may lead to internal damage such as liver, lung, heart or spleen ruptures. In instances of severe trauma, the nature of the injuries can be due to crushed skulls with brain extrusion or a rapture aorta. It is essential to recognize that biological, genetic and circumstantial factors can lead to remarkable survivals, as some individuals have survived falls from great heights with minimal injuries. (16)

In transportation or road traffic incidents, immediate death is typically associated with visible severe musculoskeletal or organ damage, significant haemorrhage, airway obstruction due to blood or traumatic asphyxia caused by chest compression from vehicle impact. Delayed fatalities may arise from ongoing bleeding, secondary haemorrhages, renal failure due to hypotension, extensive muscle damage, fat embolism, local infections or systemic infections, including myocardial or cerebral infarctions. (16)

Additionally, the presence of pre-existing natural diseases or intoxication must also be considered in trauma deaths, as they can have significant implications and may contribute to the cause of death or their circumstances. (16)

3.2.1 Traumatic brain injury – TBI

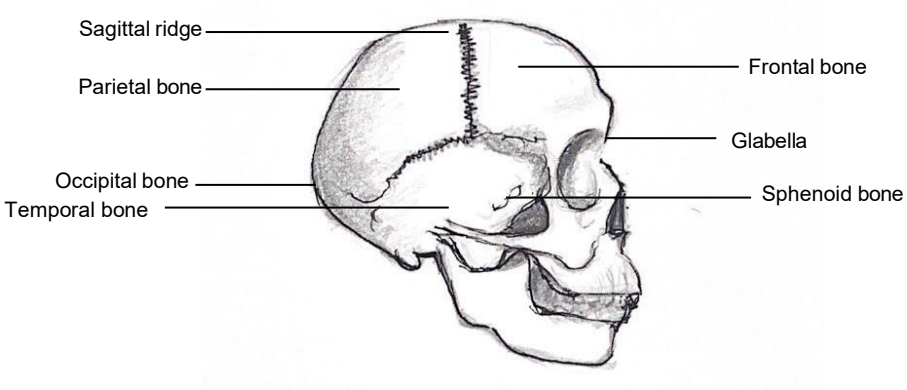


Figure 8 - Skull

The thickness of the cranium in adults exhibits variability across different regions, with thinner areas being supported by more robust structures such as the petrous temporal bone, greater wing of the sphenoid, sagittal ridge, occipital protuberance and glabella. The more susceptible regions include the parietotemporal, lateral frontal and lateral occipital zones.

It is typically not the skull fracture itself that poses a life-threatening risk, but rather the transmitted force that affects the contents of the cranium. A skull fracture serves as an indicator of the severity of the impact sustained by the head. It is uncommon for a sufficiently severe head injury to result in a fracture without also causing some form of intracranial effect. In most instances, the presence of a fracture skull signifies a significant insult to the head, which may also involve injury to critical intracranial structures, rather than the fracture being inherently life-threatening. However, there are exceptional cases where the fracture can lead to serious complications, such as when it disrupts a meningeal artery, resulting in meningeal haemorrhage. Additional complications can arise, including meningitis or the formation of a brain abscess. While conditions such as bleeding or infected scalp injuries, depressed fractures, meningitis and significant meningeal haemorrhage can be fatal, it is often the damage of the brain substance itself that proves lethal in most severe head injuries.

The brain can sustain injuries through direct intrusion from foreign objects or skull fragments in cases of compound fractures or from deformation of the brain in closed head injuries, where the injury mechanism is complex and multifactorial.

The critical factor is the change in velocity – either acceleration or deceleration – often accompanied by rotational component, which leads to brain damage. Violent relative movements between the brain and the dura mater can result in damage to cerebral tissue against sharp edges and flat surfaces of these membranes.

Traumatic brain injury (TBI) involves damage to the central nervous system resulting from external mechanical force and can be categorized through various criteria, including etiology, severity, the extent of brain tissue involvement and additional factors. [16] It is characterized by a complex pathophysiology that unfolds in two distinct phases: primary and secondary.

The primary phase involves mechanical forces disrupting the brain parenchyma and blood-brain barrier (BBB) at the moment of impact. The secondary phase involves a systemic and neuroinflammatory response mediated by immune cells and the activation of neural cells, triggering the release of cytokines, growth factors and adhesion molecules. This secondary injury can evolve over hours, days, or even months after the initial trauma, potentially leading to metabolic dysregulation and further brain injury. [2] TBI results in acute alterations to the brain's cytoarchitecture, leading to diffuse axonal injury, dendritic shearing, synaptic disruption and demyelination. These effects may be exacerbated by hematoma-induced pressure, loss of white matter and additional injury mechanisms that manifest in later phases, including necrosis, apoptosis and ongoing inflammation. [16]

TBI is clinically categorized into mild, moderate and severe, primarily based on the Glasgow Coma Score (GCS) and other clinical assessments. These tools evaluate the duration of unconsciousness, length of amnesia and resulting cognitive, behavioural or physical disabilities. The outcomes following TBI can be variable, even among similar injuries. The aim of some studies is to verify whether the outcomes are consistent with the importance of genetics in the response to trauma, particularly tin cases of TBI.

3.3. Trauma data / Epidemiology

Literature consistently highlight blunt trauma as the predominant injury mechanism. Studies show that blunt trauma accounted for more than 80% of fatalities, often associated with interpersonal violence, falls, autolysis – self-harm and traffic

accidents, with a male predominance, suggesting gender related exposure and vulnerability differences.[17]

When it comes to data in Portugal, there's no reference to trauma specifically, only the overall view of external cause of mortality in each year.

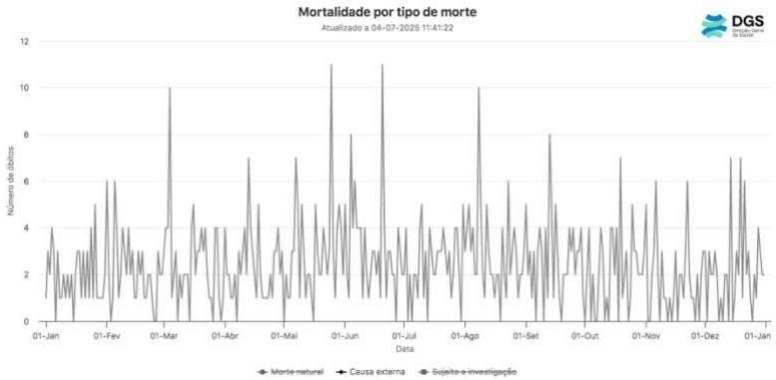


Chart 1 - External cause of mortality in Portugal (7)

Blunt injuries are frequently associated with delayed deaths, often resulting in a bimodal mortality distribution, which refers to distinct peaks in the timing of deaths, whereas penetrating trauma tends to cause immediate deaths, particularly in prehospital settings. [14]

The central nervous system (CNS) remains the leading site of fatal injury, followed by exsanguination and multiorgan failure (MOF). [17] Traumatic brain injury (TBI) and haemorrhagic shock dominate the early phases of trauma-related mortality, with MOF emerging as a significant contributor in late-stage deaths, particularly in elderly populations with comorbidities or lower initial Injury Severity Scores (ISS). ISS is a system used in trauma care to verify the severity of multiple traumatic injuries and it's based on a numeric scale where the interpretation has different levels: mild, moderate and severe injury and major trauma. [14]

Multiple interrelated factures contribute to trauma mortality patterns.

The heterogeneity of trauma is determined by multiple factors, which include mechanism of injury, anatomical injury location and biologic factors such as age and genetics.

3.4. Physiologic Aspects

3.4.1. Autopsy findings

The following information condenses some of the basic knowledge currently applied in the practice of forensic pathology and it is based on the classic reference in forensic pathology "Knights' Forensic Pathology".[16]

a. Head

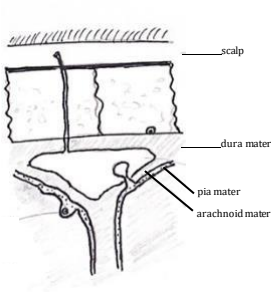


Figure 9 - Brain membranes

Head injury of any sort is recognized as having the potential to cause different forms of intracranial injury and thus TBI.

Forensic pathology has its own peculiar meningeal haemorrhage and direct brain injury. Its study is important in understanding the pathology of head injuries as well as its medico-legal implications.

The dura mater, arachnoid mater and pia mater are protective coverings of the brain. In cases of Extradural or Epidural haemorrhage, the bleeding is situated between the inner cranial surface and the dura mater. Of the three primary meningeal haemorrhages, this one is the least common. It is usually due to a skull fracture that tears a branch of middle meningeal artery which causes a high-pressure arterial bleed which separates the dura from the calvarium. Some bleeding can also occur from torn venous dural sinuses hematoma even in absence of fracture, but these tend to be small. Subdural haemorrhage is more common than extradural bleeding and occurs between the dura mater. It results from tearing of the bridging veins that lie in the subdural space. Subdural haemorrhages may develop acutely or chronically.

Subarachnoid haemorrhage (SAH) involves bleeding into the subarachnoid space and is the most common form of intracranial haemorrhage. Traumatic SAH is often associated with cortical contusions or lacerations and it's a significant cause of TBI.

In most cases of fatal cranial injury, a person's death results primarily due to the injury caused to the brain which comprises of cerebral parenchymal damage.

In closed head injury, there are more complex processes such as rotational forces acting on the head which can lead to diffuse brain injury. Also, as already mentioned, rapid decrease in motion can lead to damage on the opposite side, often the frontal and temporal lobes.

Other types of brain injury that can arise include traumatic intracranial haemorrhage which constitutes major bleeding within the brain tissue and might follow TBI or traumatic brain injury. It can be primary – happening during the impact – or secondary, which occurs due to increased intracranial pressure. It is important to note that differentiating traumatic intracerebral haemorrhage from other natural causes is critical in clinical practice. It has become evident that brain swelling is a common sequela after substantial head trauma and may be the only finding during autopsy in cases of death. This swelling also may lead to fatality from compressing important centres in the brain stem. Concussion is a term defined as a temporary functional disturbance of the brain immediately following impact and is not defined by an autopsy finding.

Detecting the cause of death after trauma injury hypoxia begins within tissue damage, while it is difficult to identify and confirm requires intensive tissue examination.

In preserving spinal trauma there is integrated functional system with the skull therefore these parts are important to consider as whole during surgery. Trauma and surgical injuries to bone structures of the cervical vertebrae have great clinical and forensic importance which can be noticed while exercising, during sports as they are caused by road traffic accidents. These injuries are caused by a combination of compression forces such as vertical compression due to a fall onto feet or head, upper cervical spine compression with the skull base may result into ring fractures around foramen magnum. Rupture of spinal ligaments associated with cervical spine move forward and backward may lead severe structural damage to the spinal canal with compression of the spinal cord.

b. Chest

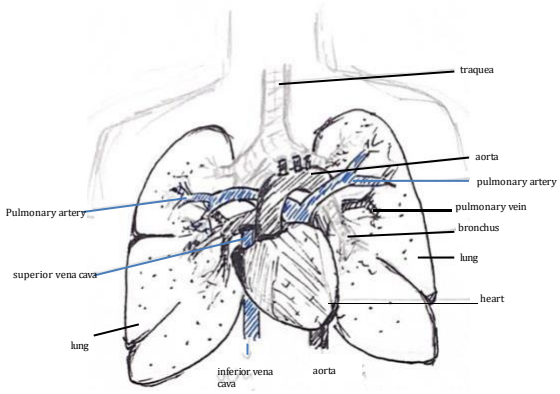


Figure 10 - Chest: heart, lungs and main vessels

From a forensic perspective, it is noteworthy that several vital organs, including the spleen and substantial portions of the liver and stomach, are anatomically situated beneath the costal margin within the thoracic cavity. Consequently, these organs are susceptible to injury from both penetrating and blunt force trauma directed at the chest.

The fundamental mechanism of respiration is contingent on the structural integrity of the chest wall. Any compromise to this rigidity, whether through severe mechanical failure of the rib cage or penetration of the pleural cavities, will significantly impair ventilation, leading to diminished air entry. A condition known as 'flail chest' can result from multiple, often bilateral, fractures of the ribs, occasionally accompanied by sternal fractures. This widespread disruption of the thoracic cage compromises its ability to expand during inspiration. The affected segment exhibits a paradoxically movement, being drawn inwards during inhalation. This phenomenon is referred to as 'paradoxical respiration'. This results in progressive hypoxia, dyspnoea, and cyanosis, with extreme presentations rapidly becoming incompatible with life. It is well-documented fact that such injuries are frequently attributed to frontal impacts, commonly sustained in motor vehicle accidents. Furthermore, displaced rib fragments have the potential to lacerate the pleura, which may result in pneumothorax, haemothorax, or both, by puncturing the lungs and forming a bronchopleural fistula. While upper rib fractures are less prevalent, they typically result from direct force or traffic accidents and invariably demonstrate evidence of haemorrhage beneath the periosteum or parietal pleura if sustained during life.

Haemothorax, a critical consequence of chest trauma, occurs when breaches in the chest wall or lung surface damage, resulting in injury to blood vessels and the pleural lining. While intercostal and mammary arteries can bleed into the pleural cavity, more massive haemorrhages often originate from larger vessels within the lung or mediastinum, with potentially fatal consequences despite minimal external blood loss.

Post-traumatic chest infections are relatively uncommon in forensic contexts due to the rapid onset of death from haemorrhage; however, they can occur with prolonged survival, particularly if introduced by contaminated weapons or foreign material, leading to conditions such as cellulitis, pleuritis, or empyema. Pneumothorax, a condition involving air in the pleural cavity, can arise from traumatic causes, such as stab wounds, creating a direct external communication. Pulmonary contusions are prevalent in both open and closed chest injuries, resulting from substantial impacts that can damage lung tissue superficially or deeply, sometimes on the opposite side of the impact ('contrecoup'). Deceleration injuries, prevalent in falls and traffic accidents, have also been observed to result in lung bruising, with rib outlines becoming discernible as lines of contusion on the lung surface. Severe bruising can manifest as subpleural blood blisters that may rupture, releasing blood or air into the pleural cavities. In severe chest trauma injuries, the potential for internal lung haemorrhage, encompassing the occurrence of hematomas and tissue breakdown, is a distinct possibility. Lung lacerations, which can even result in the detachment of lung lobes, are also observed in cases of blunt trauma. The occurrence of lacrimation at the hilum, particularly involving pulmonary veins, can precipitate severe intrapleural or mediastinal haemorrhage. Penetrating lung injuries, typically from stab wounds, can affect the lung parenchyma, large vessels, or extend to damage the heart or great vessels.

The heart is particularly vulnerable to both penetrating and blunt trauma. Stab wounds to the chest, a common method of homicide, frequently penetrate the heart. Entry can occur anywhere over the precordium or adjacent regions, usually through intercostal spaces or costal cartilage. The right ventricle, due to its larger anterior surface area, is frequently injured, but the anterior interventricular septum and left ventricle are also at risk.

Wounds to the right ventricle are often more dangerous than those to the left, as the muscular 'self-sealing' effect is less pronounced. Transfixing wounds, where the knife enters and exits the heart, are common. Blunt cardiac trauma is predominantly observed in traffic accidents, falls from height, and assaults, but any significant impact can cause fatal cardiac damage, sometimes without external thoracic injury or bony cage damage. Cardiac injuries typically affect the anterior aspect of the heart, particularly the right ventricle, although posterior bruising can occur if the heart is compressed against the spine. Injuries range from superficial epicardial bruising to extensive lacerations. Bleeding can accumulate within the pericardial sac, originating from the heart's surface or cavities, or from the great vessels. If this haemorrhage is not contained or can escape into the pleural cavities, mediastinum, or abdomen, death from exsanguination may result. Alternatively, 'cardiac tamponade' can occur when blood accumulates in the pericardial sac faster than it can be drained, or if the exit foramen becomes occluded by clot. This increased pressure impedes diastolic filling of the atria, reducing cardiac output and systemic blood pressure, ultimately leading to death if unrelieved.

In terms of great vessels, the aorta stands out as the most vulnerable major vessel, frequently sustaining injury during deceleration trauma, such as the encountered in road traffic accidents, aviation incidents and falls from significant heights. This vulnerability arises from the thorax’s abrupt deceleration, wherein the reactively mobile heart attempts to maintain its momentum, inducing severe traction at the root of the heart. A common sequela of this mechanism is the partial or complete rupture of the aorta, particularly in the descending portion of its arch. In falls, some authors believe the injury is attributed to the caudal displacement of abdominal and thoracic viscera upon impact with the ground, while others say that it is potentially linked to a sudden elevation in intra-aortic pressure. Rupture characteristically occurs approximately 1.5cm distal to the ligamentum arteriosum, the vestigial remnant of the ductus arteriosus. The lower thoracic aorta, rigidly anchored by the anterior longitudinal ligament to the dorsal spine until the terminal curvature of the arch, presents a biomechanical weak point at this juncture, leading to transection that can appear remarkably like a surgical infusion, often annular and perpendicular to the aortic axis.

While the pulmonary artery is less susceptible to blunt trauma than the aorta, it can be compromised in assaults involving stamping or steering wheel impacts, typically associated with depressed rib cage – which is the most common chest wall abnormality and its due to a genetic variation - or sternal fractures.[7] The great vessels are also frequently implicated in penetrating injuries; most notably stab wounds. [16]

c. Abdominal

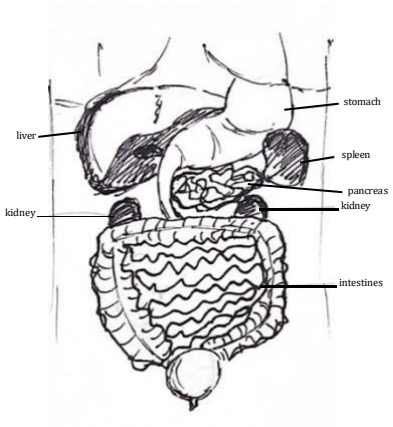


Figure 11 - Abdominal: stomach, liver, spleen, pancreas, intestines, kidneys

The extensive surface area of the anterior abdomen occupied by the intestines makes them a primary target for perforation, potentially leading to chemical or infective peritonitis. The liver and, particularly, the spleen are prone to significant haemorrhage, resulting in hemoperitoneum. Intestinal and mesenteric injuries are also common, often presenting as multiple lesions due to the overlapping arrangement of the intestinal coils and their mesentery. The stomach, offering some protection by the rib margin, is less frequently penetrated from abdominal trauma but can be involved in thoracic stab wounds that traverse the diaphragm downwards. Renal injuries are less common from anterior thrust, typically occurring from posterior assaults.

Closed or blunt abdominal trauma is a prevalent occurrence, with the liver, intestines, spleen and mesentery being the most vulnerable organs. Regardless of the mechanism, several features may manifest, including bruising of the abdominal wall, both superficial and deep, which can extend diffusely from the impact site. Importantly, severe or fatal intra-abdominal injuries may be present without any external cutaneous markers.

Extensive bleeding into the peritoneal cavity, typically from solid organ rupture or mesenteric injury, is a critical concern. Bruising or rupture of the stomach and diaphragm can occur, with the stomach being susceptible to laceration from forceful upper abdominal blows, especially when distended.
The intestines and mesentery are frequently compromised, with the duodenum and jejunum particularly vulnerable to transaction when compressed against the spine. Mesenteric lacerations are not uncommon in traffic accidents, potentially damaging or occluding local arteries.
The colon is injured less frequently, unless the abdominal trauma is severe.
Liver rupture is also a frequent sequela of severe abdominal trauma, falls or crush injuries. Hepatic lacerations can vary from superficial subcapsular tears to complete transection, often on the convex upper surface, with subcapsular haematomas potentially leading to delayed collapse or death.
The kidneys, being retroperitoneal, are less prone to frontal trauma but can be injured by blows to loin, with perirenal haemorrhage being more common that direct organ injury. Various degree of kidney damage, from comminuted pulping to superficial lacerations, can be observed. Vessel damage can occur, with the right suprarenal artery statistically more often involved in traffic accidents in the UK due to prevailing traffic direction.

The most common fatal outcome of intra-abdominal trauma is haemorrhage, with the spleen and mesentery being the most prolific sources, while liver bleeding tends to be slower unless major vessels are involved. The accumulation of blood in the abdomen, unless from an aortic injury, requires time to become clinically significant.

3.5 Inflammatory response to trauma

Haemorrhages may be external as lacerations and incised wounds or bronchi and trachea as well as oronasal passages, ears, vagina, urethra, or rectum internally. Internal bleeding describes contusions, which are blood seepages into tissues due to damaged blood vessels, as well as free bleeding into body cavities such as pericardium, peritoneum, pleura, and cranium. The amount of blood loss presents different findings, from petechiae caused by venule rupture to massive haemorrhages from a ruptured aorta.

Though, not only the total volume influences lethality but also speed and site of bleeding. Typically, blood flow starts at the moment of injury and ceases only slightly after the moment of infliction. [16]

Traumatic haemorrhagic shock (HS) is a severe consequence stemming from injury that greatly adds to the total number of traumatic deaths while also dramatically complicating outcomes, specifically after a Traumatic Brain Injury (TBI). Together HS and TBI account for roughly half of all deaths from trauma within the first 24 hours of hospital admission.[8] The combination of substantial haemorrhage and significant trauma sets off a series of post-traumatic physiological changes, inflammatory changes, and immune system reactions that can lead to damage or death of multiple organ systems. Mechanistically, systemic inflammation and organ injury in traumatic HS involve coagulation, the complement system, impaired microcirculation, and inflammatory signaling pathways. [8]

Organs such as the liver, spleen, or sometimes the lung may be injured within their capsules following trauma, even if the covering membranes remain intact. Renal failure is a common sequel to trauma; destruction of the renal tubular epithelium can occur in cases of crushed muscle, extensive burns, or exposure to toxins like mercuric salts. Trauma can also compromise arterial or venous walls, leading to false aneurysms that may later rupture, or the formation of arteriovenous fistulas that can burst. [16] Multiple Organ Dysfunction Syndrome (MODS) is identified as one of the leading causes of late death after traumatic injury, as it was mentioned earlier, representing a state where organ injuries complicate the primary trauma damage. MODS is partly attributed to excessive or maladaptive activation of inflammatory pathways. [8]

Infections at trauma sites can involve adjacent vessels, potentially causing severe haemorrhage if the vessel wall is eroded by an abscess or cellulitis. Sepsis, defined as an "unusual systemic reaction" to infection, involves a biphasic immune response with hyper-inflammatory and immunosuppressive phases, often leading to multiple organ dysfunction and susceptibility to nosocomial infections.[16] Severe sepsis occurs when sepsis is accompanied by evidence of organ dysfunction, potentially progressing to septic shock with cardiovascular collapse, often refractory to treatment.[9] The organ failure seen in severe sepsis is similar to MODS in patients who survive severe traumatic

injuries, with studies confirming a link between systemic inflammation and Multiple Organ Failure (MOF). [8;9]

3.6 Disease and trauma

In forensic pathology, one of the most complex and contentious issues arises when a death occurs in an individual who has suffered trauma but who also has a pre-existing natural disease—or where a natural disease emerges after trauma. Establishing causality in such scenarios is often extremely difficult and requires careful pathological, clinical, and circumstantial analysis. Determining the relative contribution of trauma and natural disease to the fatal outcome is a recurrent dilemma, particularly during autopsy,

Three hypotheses must be addressed:

- Whether death was caused solely by the natural disease, independent of any trauma.
- Whether death was caused entirely by the trauma, irrespective of the pre-existing pathology.
- Whether death resulted from a synergistic interaction between the trauma and the natural disease.

The considerations mentioned above are especially pertinent in cases involving coronary artery disease, subarachnoid haemorrhage and pulmonary embolism. [16]

Coronary atherosclerosis is typically a longstanding pathology, present months or years prior to any traumatic event. Trauma itself does not cause coronary atherosclerosis, it may act as a physiological stressor capable of precipitating myocardial infarction or cardiac arrest. This is particularly true in those with already compromised cardiac function. Although direct mechanical aggravation of coronary arteries—such as plaque rupture or coronary thrombosis due to trauma—is rarely observed, indirect mechanisms play a crucial role. These include increased cardiac demand during physical or emotional stress following trauma. Contemporary studies have reported a strong link between exertion and sudden cardiac death, especially in sedentary individuals abruptly exposed to intense physical activity, likely due to plaque destabilization or platelet activation. Legally, it is well established that the victim's pre-existing condition should not interfere with forensic findings. [16]

The distinction between traumatic and spontaneous subarachnoid haemorrhage represents one of the most difficult medico-legal challenges. Typically caused by the rupture of a berry aneurysm, such haemorrhages may occur spontaneously during routine physical activities—such as intercourse, exercise, or even coughing— due to transient elevations in blood pressure and pulse rate. In cases of trauma, a critical question is whether the mechanical impact of the trauma was the direct cause of aneurysmal rupture, or merely an incidental event. The issue is further complicated by the fact that small aneurysms can rupture without any external insult, while large, thin-walled aneurysms may be more vulnerable to even modest trauma. Trauma-induced SAH may be hypothesized in cases involving neck or head rotation, potentially causing vertebral artery damage near the foramen magnum. However, the direct causal link between vertebral artery injury and fatal SAH has been highly debated, since rotational impacts can damage intracranial vessels without necessarily compromising the vertebral arteries. It is worth noting that, post-mortem angiography often fails to identify a definitive bleeding point, and autopsy findings typically reveal blood pooling at the base of the brain. The identification of ruptured aneurysms necessitates meticulous examination, particularly in unfixed brains. Fixed blood is more difficult to remove from the subarachnoid space, and meticulous dissection under continuous water flow is often required to visualize aneurysmal ruptures. [16]

Pulmonary embolism exemplifies the complex interplay between trauma, surgery, immobility, and underlying venous thromboembolism. While deep vein thrombosis (DVT) is often attributable to post-traumatic immobility, it may also arise spontaneously, even in the absence of trauma or surgery. The causal relationship is strongest when the embolism occurs within two weeks of the precipitating event but becomes more speculative beyond 30 to 90 days. Forensically, the presence of fat or bone marrow emboli in the pulmonary vasculature provides strong evidence of ante-mortem trauma, as such emboli require active circulation to reach the lungs. [16]

Osteogenesis imperfecta (OI) is a bone disease and a heritable disorder of connective tissue. It is caused by a polymorphism is genes COL1A1 and COL1A2 that encode type I collagen. OI is a highly variable disorder, with severity ranging from perinatal lethality to a mild predisposition to fractures. The relationship between these gene variants and trauma is direct and profound, primarily manifesting as bone fragility and a high susceptibility to fractures.

Individuals with OI often sustain fractures with minimal or no trauma, affecting mostly bones in extremities. [5;15]

In perinatally lethal OI, severe abnormalities are evident at birth. Infants often succumb to pulmonary insufficiency due to multiple rib fractures and a “flail chest” caused by fragile ribs.

In legal medicine, it is imperative to make a differential diagnosis between fractures due to OI and non-accidental trauma or child abuse, given that, at plain sight, both can be characterized by identical fractures. It’s essential to examine the patient’s clinical records and family history. In cases of non-accidental trauma with unexplained fractures, genetic testing for COL1A1 and COL1A2 should be considered. [15]

The forensic assessment of deaths involving both natural disease and trauma requires a nuanced, objective, and evidence-based approach, according to the autopsy findings and clinical history.

Determining whether death was a direct consequence of trauma, an inevitable outcome of disease, or the result of their interplay lies at the heart of forensic pathology. In all cases, the meticulous examination of anatomical, clinical, and circumstantial data remains the cornerstone of establishing a just and scientifically sound conclusion. [16]

3.7 Genetics

At this point, a brief review of fundamental concepts in genetic, genetic polymorphisms and human biology is needed to contextualise the subsequent discussion.

The human organism consists of a complex multicellular system in which cellular functions are governed by a genome made up of DNA. Organized in genes, the information contained in these sequences results in specific phenotypic traits and physiological responses.

Genetics encompasses the study of heredity and variability in organisms. Within the genome, composed with nucleotides, polymorphisms can occur, which are natural variations in the DNA sequence. Single nucleotide polymorphisms (SNPs) are one of the classes of polymorphisms that can occur, which constitute common genetic variants, often associated with an increased risk of disease.

Models that study SNP polymorphisms have been utilized to examine the diverse responses of individuals to injury and other inflammatory or infectious stimuli. According to the prevailing definition, a SNP is considered to be a position of single base pair variation in the genomic DNA, whenever there are alternative forms of the sequence with a frequency greater that 1%. Consequently, the term SNP does not include numerous other single base substitutions where the least common allele is present at a frequency of 1% or less, nor other variations, such as insertion or deletion polymorphisms. It is imperative to differentiate between SNPs and disease-causing mutations, which are comparatively rarer but exhibit higher penetrance (e.g., sickle-cell anaemia). It is important to note that while SNPs themselves do not originate disease, it is possible they can to affect the risk of developing a disease (for example, diabetes mellitus, asthma), or the severity of a disease or condition (for example, mortality due to sepsis, the incidence of atherosclerosis in cardiac transplant recipients). While a significant amount of research has been dedicated to SNPs, it is important to recognise that microsatellite and insertion/deletion polymorphisms represent other forms of genetic variation that may be utilised to characterise an individual's risk for post-traumatic sepsis, organ dysfunction or death.[3] Concerning traumatic injury, the host initiates a cascade of biological processes aimed to mitigating damage and promoting repair. Individual's genetic, specifically the presence of relevant polymorphisms, can modulate these responses. Research on the genetic factors influencing recovery from traumatic injuries has been predominantly focused on traumatic brain injury (TBI), with limited data available for other traumatic injuries in general. Consequently, the following discussion is focused on studies and findings specific to TBI. [2; 11]

It has been described that TBI is a significant cause of death characterized by heterogenous patient outcomes influenced by genetic factors. [2; 4; 11]

Regarding the information collected from some of the analysed articles, a list of all the polymorphisms highlighted in the investigations is presented, with those mentioned in more than one article appearing in bold.

Table 2 – Genes involved in TBI

Genes
APOE (E2, E3, E4)
TNFα
IL-1
IL-6
IL1RN
COMT
BDNF
NOS3
ACE
TP53
MAPT
TGFβ
NOS3
mtDNA haplogroup K
COL1A1 e COL1A12

4. Results and Discussion

4.1. Inflammatory response and trauma-related death

After conducting a broad review of the literature, it was found that haemorrhagic shock occurring in isolation, is an uncommon occurrence. It has been demonstrated that after a traumatic event, it functions as the catalyst for a complex systemic failure, which ultimately results in multiple organ dysfunction and death. The initial phase, is characterized by a decrease in circulating and insufficient perfusion, often progressing to a systemic crisis. This systemic crisis, although it may not result in immediate fatality, is a progressively fatal event. It has been established that, fatality arises not from the initial injury itself, but rather from the organism excessive reaction to it.

It is important to note that a considerable number of trauma victims do not immediately succumb to their injuries; instead they deteriorate over several days, as organs begin to fail sequentially. This delayed mortality is of particular concern in the interpretation of both clinical and forensic cases. [16;17]

In the forensic context, the transition from haemorrhage shock to multiorgan failure has the potential to obscure the underlying cause of death. Nevertheless, an analyse of clinical timeline of these events, frequently suggest that the crucial moment lies in uncontrolled early blood loss. Additionally, genetic variation in the immune and coagulation responses is likely to play a determinant role in survival outcomes. [12] It has been referred that, among patients exhibiting analogous injury patterns and comparable resuscitative efforts, some exhibit rapid deterioration into MOF, whereas others demonstrate recovery. This raises important questions regarding genetic polymorphisms within inflammatory pathways and regulation. They may assist in guiding future medico legal interpretations.

4.2. The interplay between disease and trauma

A particularly complex aspect of forensic pathology is the interpretation of deaths, where both natural disease and trauma co-exist. While the theoretical mechanisms by which this occurs are thoroughly established, ascertaining the causal factor that determined the outcome remains a challenge. Consequently, the forensic process entails not only the identification of present elements, but also the evaluation of their contributions, understand how trauma can amplify latent vulnerabilities, or how pre-existing pathologies can diminish the total impact of trauma.

This diagnostic grey zone includes coronary artery disease, subarachnoid haemorrhage and pulmonary embolism, as it was discussed earlier. [16] It is acknowledged that while a blow to the chest may not result in the rupture of a coronary artery, it has the potential to trigger a myocardial infarction in an individual suffering from severe atherosclerosis. Similarly, a ruptured aneurysm may be spontaneous during physical effort or because of stress associated with a traumatic event. In circumstances such as these, trauma may not be the primary cause of death; however, it is rarely incidental. The analysis of Knight's Forensic Pathology [16] demonstrated that the causal causation is frequently shared among individuals. They also concluded that trauma and disease do not act isolated; instead, they establish a synergistic vulnerability.

In practical terms, this renders to three significant interpretive questions:

- Whether the individual would have died at the time in the absence of the traumatic event;
- Whether the disease, in the absence of any trauma, would have been sufficient to cause death at that time;
- Whether the two processes, disease and trauma, contributed significantly to the fatal outcome.

The questions are not merely theoretical in nature. The influence of these factors is evident concerning legal responsibility insurance decisions and the broader public heath understanding. In cases where a precise diagnosis is not achievable, the opinion of the pathologist is of significant value. This opinion must be bases on objective analysis. However, it is also vital that considers the clinical history. Thus, the forensic pathologist is expected to consider the broader context beyond the limitations of isolated findings. In practical terms, in the context of traumatic brain injury, the occurrence of a ruptured aneurysm cannot be disregarded as spontaneous, without due consideration of the potential role of blunt force, elevated blood pressure or emotional distress in triggering the rupture. Not forgetting to take into account the potential role of blunt force, high blood pressure, or emotional distress in triggering the rupture. [16]

Another condition mentioned in this study, illustrative of these complex interactions, was osteogenesis imperfecta (OI) – a genetic disorder characterised by the imperfect production of collagen formation, which compromises bone strength and structural integrity. Individuals with OI may suffer extensive bone injury due to minimal trauma and these can be fatal when they occur after events that would be non-lethal in healthy individuals. From a forensic point of view, particularly in cases where suspicion of abuse may arise, a precise and careful analysis is required, to distinguish between inflicted trauma and the patterns of pathological fracture. Considering the questions stated before, OI does not refute the role of trauma; instead it defines the context of fragility within which the trauma occurred. This issue highlights, once again, the importance of considering both anatomical vulnerability and situational context in forensic investigations. [13;15]

Overall, this topic results in broader considerations about how “death from trauma” is defined. In certain cases, trauma itself is undoubtedly lethal. In other, it acts as a catalyst, sometimes silently, transforming a stable illness into a terminal disease. Recognizing these complexities is not only important from medical and legal standpoint, but also ethically crucial, thereby ensuring that each case is fully understood.

4.3. Genetic polymorphisms in trauma response

Trauma response is governed, not only by mechanism of injury, but also by genetic variations, which modulates inflammation, healing and repair processes. SNPs in different genes have been shown to directly influence that response.

As previously mentioned, genetic research related to traumatic injuries has not been covering all types of injuries, with most studies and specific findings focusing on TBI. Among the most studied inflammatory markers, cytokines and their genetic polymorphisms have been linked to differences in clinical outcomes following TBI.

The tumour necrosis factor alpha (TNFα), a cytokine that regulates neuronal death and synaptic plasticity, has a well-known polymorphism – the TNFα - 308 A allele - which is associated with poorer outcomes due to elevated expression of this pro-inflammatory molecule. This polymorphism translates in a higher expression of TNFα and, consequently, a higher possibility to severe disability and unfavourable outcomes in TBI survivors. In different analysed studies, it also suggests that it did not impact significantly in mortality. [2;4]

Similarly, interleukin-1 (IL-1), particularly its IL-1α and IL-1β isoforms, plays a key role in amplifying inflammation and inducing fever, following injury. The IL-1β + 3953 T allele has been correlated with increased intracranial pressure and greater injury severity in TBI patients. [10]

Polymorphisms in the IL1RN gene have also been target of study in relation to TBI. IL1RN2 allele has been associated with higher rates of haemorrhagic contusions, which results in less favourable outcomes following TBI. [3]

Interleukin-6 (IL-6) production is stimulated by TNFα and IL-1. It represents another cytokine with dual effects: while it can exert neuroprotective actions, it also contributes to neurotoxicity under certain conditions. The IL-6 – 174 GG genotype has been linked to more favourable outcomes post-injury and could be associated with a lower risk of death following TBI. Whereas individuals with the IL-6 – 572 CC genotype might exhibit increased susceptibility to concussion. [2;12]

Apolipoprotein E (APOE) is, to date, the most extensively studied protein in the context of TBI. It plays a pivotal role in neuronal repair, growth and modulating the inflammatory response.

E2, E3 and E4 are three common isoforms, but the APOE4 allele is of particularly interest, which has been consistently associated with worse clinical outcomes, including prolonged hospital stays, greater injury severity and an increased risk of developing post-traumatic Alzheimer's disease. Some studies show that individuals

with an APOE4 allele were less tolerant to increases in cerebral perforation pressure which can be related to mortality risk and it was also linked to higher fatality rates. [2;4;6]

Transforming growth factor beta (TGFβ), a regulator of inflammation and tissue repair, during the acute phase of TBI, due to the inhibition of IL-1, IL-6 and TNFα production. The TGFβ - 509 T allele may confer greater neuroprotection in the outcome of TBI. [2]

Beyond cytokines and APOE, several other genetic polymorphisms have demonstrated relevance in TBI prognosis. For instance, the tau protein (MAPT), that is involved in stabilizing microtubules, when hyperphosphorylated, is a hallmark of various neurodegenerative disorders such as Alzheimer's disease and chronic traumatic encephalopathy (CTE). Following TBI, elevated MAPT levels have been detected in cerebrospinal fluid and plasma, which corelates it with the severity of the injury and the outcome. Additionally, the Tau Ser53Pro TT variant may enhance the risk of recurrent concussions. [2]

The NOS3 gene, encoding endothelial nitric oxide synthase, is essential for regulating cerebral blood flow. The -786 C allele of NOS3 has been associated with reduced perfusion following TBI, potentially worsening outcomes. [2;11]

The catechol-O-methyltransferase (COMT) gene, which modulates dopamine levels in the prefrontal cortex, also plays a role in cognitive outcomes after TBI. The Val158Met polymorphism is linked to high enzyme activity and lower dopamine levels. It appears to impact executive functioning, with Val allele carriers showing poorer performance, following TBI. [2;4;11]

Additionally, the angiotensin-converting enzyme (ACE), known for its role in blood pressure and cerebral perfusion regulation, presents a D/D genotype that has been linked to worse cognitive outcomes post-injury. [2]

Brain-derived neurotrophic factor (BDNF), a key modulator of synaptic plasticity and memory, is also relevant; individuals carrying the Val66Met polymorphism may experience reduced cognitive performance following TBI. [2;4;11]

Furthermore, tumour protein p53 (TP53), a critical regulator of apoptosis, is implicated in injury outcomes as well. The Arg/Arg genotype of TP53 has been associated with more severe prognoses in cases of major TBI. Studies don't state a direct correlation with mortality after TBI, but it states a bad outcome, which can be related with higher mortality. [2;11]

Lastly, mitochondrial genetics is an emerging field in TBI research. Given the mitochondria's central role in energy production and oxidative stress regulation, certain mitochondrial DNA polymorphisms have shown prognostic potential. Specifically, the mtDNA haplogroup K has been linked to better recovery outcomes, potentially lessening the negative effect of aging. while the A10398G variant appears to influence recovery trajectories in a sex-specific manner. [2]

Regarding trauma in general, COL1A1 and COL1A2 genes are responsible for encoding the alpha-1 and alpha-2 chains of collagen type I, respectively. Collagen it's a very important protein present in bones and other tissues. Abnormalities in these genes make the individual more prone to fractures in bones and progressive deformity, which can increase risk of mortality. It is important to note that no specific polymorphism is described in bibliography. [15]

The following table (Table 3) presents these findings in a more summarized format, where protective suggest a protective effect against severe trauma outcomes and risk factor suggest a negative effect, increasing risk of complications and even death.

Table 3 – Studied polymorphisms and its characteristics

Gene / Polymorphism	*Main Function / Mechanism*	*Impact on Trauma Outcome*	*Interpretation*
IL-6 -174 GG	Regulates inflammation; anti-inflammatory profile	Linked to better neurological recovery	Protective
TGF-β -509 T	Inhibits pro-inflammatory cytokines (IL-1, IL-6, TNF-α)	Promotes neuroprotection and healing	Protective
mtDNA Haplogroup K	Enhances mitochondrial resilience to oxidative stress	Associated with improved recovery after TBI	Protective
TNF-α -308 A	Increases TNF-α production; promotes inflammation	Linked to worse neurological outcomes	Risk factor
IL-1β +3953 T	Amplifies inflammatory response	Associated with increased intracranial pressure	Risk factor
*IL1RN*2*	Alters regulation of IL-1	Correlated with haemorrhagic brain contusions	Risk factor

IL-6 -572 CC	Modulates inflammatory profile	Linked to higher susceptibility to concussion	Risk factor
APOE ε4	Modulates neural repair and lipid transport	Associated with poor recovery, post-traumatic dementia	Risk factor
MAPT Ser53Pro TT	Involved in microtubule stability	Increases risk of chronic traumatic encephalopathy	Risk factor
NOS3 -786 C	Reduces nitric oxide production; affects cerebral blood flow	Linked to worsened perfusion and outcome	Risk factor
COMT Val158Met (Val/Val)	Affects dopamine breakdown and cognitive function	Associated with poorer executive functioning post-TBI	Risk factor
ACE D/D	Increases vascular tone and resistance	Correlated with poor cerebral perfusion	Risk factor
TP53 Arg/Arg	Regulates apoptosis and DNA repair	Related to poor prognosis in severe TBI	Risk factor

These findings emphasise the relevance of considering genetic susceptibility when interpreting traumatic deaths, highlighting the potential benefits of incorporating these molecular profiling into forensic assessments. As already mentioned, molecular profiling would help to improve the understanding on how individual variations can influence in trauma response and mortality risk among individuals.

4.4. Medico legal relevance

Trauma, pre-existing disease and genetic predisposition, taken together, constitute a deeply complex, but increasingly pertinent matter within forensic medicine.

The bibliography reviewed in this work, which includes genetic findings, clinical patterns and autopsy findings, allow us to conclude that there are no two cases of traumatic death are the same and the biological context of each individual is of profound importance.

Recognising the influence of pre-existing conditions, such as those previously discussed, is of extreme importance. It is essential not only for an accurate determination of the cause of death but also for the correct interpretation of legal responsibility. This underscores the fundamental role of forensic pathologists, who are responsible for combining pathological evidence, medical history and physiological plausibility.

It is also important to recognize that trauma outcomes are not purely mechanical. Genetic findings, including polymorphisms in cytokines, coagulation factors, neuroprotective proteins, allow us to understand that individual biological variability influence both the immediate response to injury and the subsequent recovery process. These findings also suggest genetic susceptibility to trauma, whereby distinct profiles may exhibit resistance, while others appear to be predisposed to deterioration, multi-organ failure or death.

In this context, it is imperative that the field of forensic science evolves beyond conventional approaches such as histopathology, embracing molecular and genetic tools that can elucidate the underlying causes of fatal injuries, particularly in cases where the initial prognosis would have indicated a positive outcome. Furthermore, it is extremely important to understand why two individuals with identical traumatic events can exhibit significantly divergent outcomes. The issue assumes particular significance when the legal process needs a clearer causal narrative, which can be facilitated using genetic and physiopathological markers.

Additionally, these findings provide a better understanding of delayed traumatic deaths. In cases where a HS leads to secondary inflammatory cascades and eventual MOF, trauma remains the initiating event, even if death occurs days or weeks later. In these scenarios, meticulous forensic analysis is imperative to ensure the causality of legal record, especially when clinical records complicate the interpretation.

This multifactorial and complex understanding offers new pathways for the scientific development of forensic science, medico-legal practice and even trauma prevention. Personalized forensic evaluation is encouraged, assessment can bring many advantages, combining genetic screening, systemic analysis and contextual insight to ensure more accurate and defensible conclusions concerning the cause of death in each case.

4.4. Limitations and future perspectives

Despite the considerable progress in molecular biology, pathophysiology and forensic science, there are still some limitations that prevent a full understanding and application of this knowledge.

First of all, the existing literature on genetic polymorphisms and traumatic outcomes is supported by studies in animal or hospital data or studies with small cohort, with a limited minimum demographic diversity. This limits the applicability of research findings, particularly to forensic samples, which may include victims from a very different genetic, environmental and ethnic backgrounds. The underrepresentation of certain populations in genomic studies has a limited effect on the identification of specific genotypes, whether protective or a risk factor, that are relevant to traumatic deaths.

Furthermore, there persists a lack of standardisation in the forensic application of genetic biomarkers. Despite a significant number of polymorphisms having been associated with positive or negative outcomes in TBI or HS, there is currently no agreement on which markers are forensically viable. The absence of these regulatory guidelines and reference standards has a detrimental effect on the regular use of genetic evidence in forensic autopsy contexts. [6]

In practical terms, many studies discuss prioritizing correlation over causation. For example, although certain polymorphisms may occur more frequently in those who die from trauma, determining direct and accurate correlations remains a challenge. In forensic medicine, establishing causality is of utmost importance, however, it presents a significant challenge for the reliable interpretation of results.

Moreover, access to molecular and genetic tests remains limited due to high costs, lack of technical skills and equipment restrictions. [10] Despite the availability of the technology in question, the incorporation of genetic and molecular discoveries in forensic cases requires a multidisciplinary collaboration, which is still evolving in various ways.

On the other hand, in the future there is considerable potential for the development in forensic medicine, with more genetic analysis and data, for a more accurate interpretation of the cause of death. Large-scale population-based studies that incorporating traumatic death and genetic polymorphisms would significantly increase the reliability of biomarkers and promote the development of forensic models. [2;3;11]

International databases for trauma related polymorphisms, along with the development of guidelines and protocols, have the potential to standardise and validate the role of genetic evidence in cases of traumatic deaths. To achieve this potential, investment in training, equipment and interdisciplinary collaboration between forensics pathologists, biologists and legal professionals is essential. Thus, the current challenge is to establish a connection between the emerging scientific knowledge in this field and its practical, ethical and legal implementation. These perspectives are still in early development, however, have great potential. Portugal is no exception, and all the conditions mentioned above are also applied.

The main need is to advance in the research field. Searching and reviewing the bibliography for this study, no studies, related to this theme and area, were found to have been conducted in Portugal. Thus, it is needed a significant investment in genetic and molecular analyses related to trauma, investment in its equipment and strengthen interdisciplinary training for forensic experts. [6] A genetic database centered in biomarkers related to trauma is required, in order to enhance research and, later, evolve to applying those findings in post-traumatic investigations. Lastly, ethical and legal frameworks must evolve alongside these scientific developments, such as, consent, data protection and interpretation standards. [5,8]

In general, new doors may be open for forensic medicine specially in Portugal. With the right investments and research, a better understanding of genetic individual variability and trauma could improve the determination of the cause of death, advancing forensic and legal medicine.

5. Conclusion

The present study aimed to explore the complex relationship between trauma, genetic variability, physiopathological processes and pre-existing conditions within the scope of forensic pathology and legal medicine. A comprehensive review of the existing literature reveals that understanding trauma-related mortality is incomplete without considering the biological variability of each individual.

The interaction between inflammatory response, haemorrhagic shock and multi organ failure it an important factor in traumatic death. The duration, severity and anatomical location of injury are crucial for the risk of mortality, and these effects are worsened by genetic and immunological factors.

It is important to note that these mechanisms do not exhibit uniformity among individuals; instead they are shaped by genetic polymorphisms that influenced immune

regulation, coagulation pathways, neuroprotection and metabolic responses. The presence or absence of specific alleles has been shown to significantly alter the course of recovery or fatality. As these findings indicate, trauma should be assessed not only in terms of its physical injury, but also as a biologically modulated event with deeply individualised outcomes.

Another crucial challenge is to distinguish the contributions of trauma and pre-existing disease to a fatal outcome. Cases involving coronary artery disease, subarachnoid haemorrhage, pulmonary embolism or osteogenesis imperfecta highlight the need for a contextual approach that goes beyond simplistic causal hypotheses. Therefore, a forensic pathologist must encompass not only the identification of the injuries sustained but also an analysis of influence of the individual's history.

The growing application of genetic analysis in the investigation of traumas represents a new domain for forensic science. Nevertheless, there are significant limitations in this research. There remains low investment in the field, a lack of large-scale forensic genetic studies in humans, an absence of standardised protocols and limited access to advanced testing techniques. These barriers emphasis the need for ongoing research and substantial investment.

In future perspectives, research should expand genomic databases with trauma specific data, the validation of forensic biomarkers in diverse populations and further investigations related to trauma, even for clinical subjects, regarding trauma prevention and response, in order to reduce traumatic mortality rates.

In conclusion, this study validates the need for a comprehensive approach when it comes to traumatic deaths.

It is essential, to understand the complex nature of the subject, which includes external injuries, internal conditions, genetic factors and complex biological events involved in trauma outcomes. The future of legal medicine and forensic pathology relies in its capacity to combine these aspects, with the aim of objectively explaining the manner of death and its underlying causes.

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7. Attachments

Attachment 1 - Abbreviations and its meanings

Abbreviation	*Meaning*
TBI	Traumatic brain injury
GCS	Glasgow Coma Score
CNS	Central nervous system
MOF	Multiple organ failure
ISS	Injury Severity Scores
SAH	Subarachnoid haemorrhage
HS	Heamorrhagic shock
MODS	Multiple organ dysfunction syndrome
DVT	Deep vein thrombosis
OI	*Osteogenesis imperfecta*
DNA	Deoxyribonucleic acid
SNP	Single nucleotide polymorphism