

Review

Cartilage Tissue in Forensic Science—State of the Art and Future Research Directions

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Abstract: Cartilage tissue performs many functions in the human body. The diseases and injuries affecting it are prevalent due to its slow regeneration rate. However, cartilage tissue is exceptionally important for its auspicious use in forensic medicine due to its slow postmortem degradation rate. The presented review summarizes the latest research on cartilage tissues and their current and potential applications in forensic science. It also describes the most important studies on using cartilage and its microscopic and macroscopic analyses to estimate the deceased age and determine postmortem interval (PMI) values and the crime weapon. Additionally, the review describes attempts to isolate DNA from cartilage tissue for individual identification. The review also mentions recent, less abundant studies on the cartilage in forensic toxicology and genetics. It points out further directions and prospects for research development on cartilage tissue and its promising use in forensic medicine

Keywords: age estimation; computed tomography; costal cartilage; forensic genetics; forensic toxicology; postmortem interval



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1. Introduction

Cartilage is a unique type of connective tissue, devoid of nerves and vessels, nourished by the diffusion of nutrients. It consists of a cellular component and an extracellular matrix (ECM). The extracellular matrix consists of polysaccharides, fibrous proteins, and water. Cartilage tissue can take the form of hyaline, fibrous, and elastic cartilage [1]. Hyaline cartilage is translucent, hard, and surrounded by a special membrane-perichondrium. It forms articular surfaces [2]. Human costal cartilage (or human rib cartilage) connects the ribs to the sternum and plays a crucial role in the structural stiffness of the ribcage [3]. Fibrous cartilage, among others, is present in tendons and ligaments, where the intertwined collagen fibers protect the cartilage against tearing and excessive pressure. The calcified fibrous cartilage in the tendon and its connection to the bone provide additional support [4]. Elastic cartilage consists of elastic fibers, which are insoluble ECM components. It comprises dynamic connective tissues such as skin, arteries, lungs, and ligaments. Its function is to give tissues some elastic recoil [5].

The importance of cartilage in medicine is clearly marked, as its diseases affect millions of people worldwide. One of the most common cartilage diseases is osteoarthritis, which is currently considered incurable and, therefore, intensively researched [6]. However, the role of cartilage in forensics is not sufficiently explored. Due to the properties and the postmortem changes in the cartilage tissue structure, it is suggested that cartilage tissue analysis could be incorporated into forensic practice. According to Banasr et al. [7], changes

in cartilage and bone tissue are present in 53% of autopsied bodies presenting stab or incised wounds. Cartilage damage is more common than bone damage, which presents a significant advantage over bone tissue [7]. Cartilage should also be considered a source of important anatomical variations helping identify the deceased. In addition, the knowledge of cartilage's anatomical variations could help criminologists distinguish between changes of criminal origin and these naturally occurring. An example of such a cartilage lesion is the apophyses symmetrically arising from the posterior margins of the thyroid laminae described by Porzionato et al. [8].

The review aims to present cartilage as a valuable material in forensic medicine and related sciences, forensic toxicology, and genetics. The review also presents and summarizes the most important research on using cartilage to estimate the individual's age and determine the postmortem interval (PMI).

Furthermore, the presented review describes the methods of imaging and diagnosing cartilage defects to determine the cause of death and the potential murder weapon. As postmortem imaging is becoming increasingly important, particular attention is paid to using computed tomography (CT). In modern forensic medicine, it is possible to visualize changes in the thyroid cartilage and hyoid bone of suicide victims, fractures of the cervical vertebrae in the great height fall and jump victims, or changes in stabbing victims [9]. In the times of developing CT imaging methods and their use in forensic medicine, in some cases, it seems necessary to use CT in addition to standard autopsy in order to determine the cause of death. Such an interdisciplinary approach was presented by Amadasi et al. in the case combining hanging and falling, known as the 'hangman's fracture' case [10].

2. Methods

We performed a literature search using available scientific databases such as Pubmed, Embase, Google Scholar, and Scopus using the following keywords: "cartilage tissue and forensic science". We searched for original research papers, case studies, and technical reports, no older than 52 years. The returned results were searched for studies on cartilage collected from deceased persons, with particular emphasis on attempts to use cartilage for age estimation, PMI estimation, postmortem gene analysis, and toxicological examination. Articles focusing on measuring cartilage properties using novel imaging methods were also included in the final search score.

3. Age Estimation

One of the most promising forensic applications of the cartilage tissue appears to be age estimation, as changes in cartilage occurring with time may be an important indicator of the individuals' age. Cartilage fibrillation is one such indicator. In 1973, Meachim and Emery [11] discovered that articular cartilage fibrillation develops in all synovial joints in the arm and hip in the second decade of life. The process begins circumferentially and in characteristic places, e.g., the mid-zone of the inferomedial segment of the femoral head and the mid-zone of the acetabular surface, and the zone expands with age [11]. Likewise, the cartilage fibrillation process is prevalent in the ankle joint and can already be detected in young adults [12].

Other parameters, in parallel with the degree of calcification, are being evaluated. Oktay et al. [13] showed that the degree of manubriosternal joint degeneration and calcification in the second costal cartilage significantly increase with age, but its range is wide. As the authors themselves emphasized, their results might be used to support traditionally accepted age-estimation methods but not as independent indicators of specific age ranges [13]. Multislice-computed tomography (MSCT) of the sternal end of the first right rib provides some interesting information about osseous and calcic projections (OCP) occurrence in patients aged 15–30. Moskovitch et al. [14] proved that the absence of OCPs indicated that the living male subject was less than 25 years old. On the other hand, the presence of two OCPs indicated that the living female subject was more than 20 years old [14]. Assessing the degree of cartilage tissue ossification, another phenomenon related

to cartilage remodeling, is also useful in forensic medicine. The clavicle epiphysis ossification is divided into five stages: 1—non-ossified epiphysis, 2—discernible ossification center, 3—partial fusion, 4—total fusion, and 5—disappearance of the epiphyseal scar. The ossification stage classification of cartilage tissue is used in young adults and in X-ray examinations. According to Schmeling et al., the disappearance of the epiphyseal scar (the 5th stage) is observed in both sexes at the age of 26, at the earliest [15]. Furthermore, the differences in costal cartilage ossification pattern can help determine the individual's sex. The peripheral model of ossification with cartilage deposits of the upper and lower edges is observed in men, while in women, the ossification is central and of a pyramidal shape. Additionally, age estimation is possible based on the central globular foci presence in the costal cartilage. They can be observed in both sexes as of the third decade of life, but more often in women than in men (62% vs. 34%, respectively) [16]. Future forensic research should also pay attention to the ossification processes in the lungs and bronchial walls, as they may be related to the individual's age and comorbidities [17].

Several studies indicate that costal cartilage calcification differs between men and women [18–20]. The costal cartilage calcification degree assessed radiographically can be used to determine the sex of human remains. The radiographic method has numerous advantages, but most importantly, the method is quick, low-cost, and non-invasive [21]. However, reliable results were only obtained if the calcified deposits in the second to seventh costal cartilages were predominantly trabecular bone or sclerotic calcified deposits. Specimens with minimal amounts or similar amounts of trabecular bone or sclerotic deposits in the costal cartilage are not eligible for this method [22]. Calcified cartilage in femoral heads can also be used to determine the age and gender of the individual. Nielsen et al. proved that the volume and thickness of the calcified cartilage in femoral heads are age-related and different for women and men. They found that the calcified cartilage thickness and volume positively correlated with age, but in women only [23].

Cartilage calcifications analysis is valuable in forensics, especially in the case of mummified individuals, where soft tissue and bone may adhere tightly to each other, making the examination of the hyoid bone and thyroid cartilage difficult. In such cases, radiological analysis of calcified thyroid cartilage, inextricably linked with the hyoid bone continuity analysis, may be crucial in demonstrating the cause of death by strangulation [24]. However, maceration is recommended in the cases of advanced putrid decomposition and, consequently, difficulties with the discoid cartilage structure analysis [25]. In the cases with one month PMI, thyroid cartilage microscopic analysis may allow the distinction between recent and old wounds by revealing incompletely ossified fibrous tissue [26]. However, despite the relationship between thyroid cartilage ossification and age, computed tomography showed that the narrow age ranges are hard to estimate [27]. Age estimation based on laryngeal cartilage is also possible. Radiographic analysis showed a positive correlation between laryngeal cartilage age and the total score of radiopacity. Unfortunately, despite the high reproducibility of the results and the significant correlation coefficient, the method shows large inter-individual discrepancy in the same age group [28]. Moreover, the laryngo-hyoid complex presents a wide range of anatomical anomalies. Therefore, the diagnosis of traumatic lesions in this area is complex and requires excellent caution [29]. In order to use the cartilage ossification assessment method in everyday forensic practice, the results should be standardized and adapted to the individual characteristics of the deceased. The Bayesian statistics method assures such an approach. The method sequentially updates the deceased's age estimation table by including exceptional data when examining thyroid cartilage ossification [30]. Using the Bayesian statistics method significantly improves the reliability of the results of cartilage ossification assessment with CT as it blurs the error resulting from inter-individual differences in the same age group. According to Ikeda [31], the method improves the correct age classification rate for 40% of males and 35% of females. The combination of CT and the Bayesian statistics method seems to be a promising approach to age estimation [31]. Ikeda's research also showed the importance of computed tomography in cartilage analysis for forensic purposes [31]. Using CT for determining

the causes, extent, and background of third-party involvement in a neck injury may even be superior to the standard postmortem fine preparation method. Nevertheless, in the case of light cartilage-related neck injuries in young people without the complete fusion of cartilage elements, the classic postmortem fine preparation still seems to be a better choice, showing that the use of cartilage in forensics is limited [32].

Special attention should also be paid to using magnetic resonance imaging (MRI) in forensics. Compared to CT, MRI minimizes the body's, when the subject of forensic examination is a living person, or material's exposure to radiation. Ekizoglu et al. [33] described an age estimation method based on the distal radius ossification degree of the deceased using fast spin-echo proton density (FSE PD)—weighted MRI, which visualizes hyaline cartilage. However, they found the method effective only for men 15–17 years of age and women 14–16 years of age [33]. They also retrospectively assessed the ossification degree of the distal femoral and proximal tibial epiphyses using a different MRI sequence—T1-weighted turbo spin echo (T1-TSE) MRI sequence. They found a significant correlation ($p < 0.001$) between age and ossification in these areas; however, again, the method was reliable only for people 14–17 years of age [34].

The correlation between actual age and the D/L aspartic acid enantiomers ratio has been used for age estimation in forensic cases for years [35,36]. Age estimation based on aspartic acid racemization (AAR) was applied successfully to various tissues, e.g., bones and teeth [37]. Healthy teeth are preferable because caries induce protein degradation and may influence the AAR kinetics in dentine [38]. Determining the chronological age based on the racemization rate of amino acid in human dentin can be very precise, with an approximate error of 1.5–4 years and a correlation coefficient of 0.97–0.99 [39,40]. Other studies focusing on an age-dependent increase in D-aspartic acid also showed high correlation coefficients, e.g., purified elastin from arteries $R = 0.90$ [41], sclera $R = 0.92$ [42], elastic cartilage of the epiglottis $R = 0.92$ [43]. The study by Pfeiffer [44] analyzed two fractions of costal cartilage, obtaining the correlation value of $R = 0.91$ for an acid-soluble peptide fraction and $R = 0.97$ for an insoluble collagen-rich fraction [44]. However, the study by Tiplamaz et al. [45] emphasized the need to collect additional, apart from cartilage, tissue to estimate the deceased's age with the greatest possible precision. It may be skin in which, as in cartilage, the rate of aspartate racemization correlates significantly with the age of the deceased [45]. It can, therefore, be assumed that cartilage from various sources can be successfully used for age estimation using the degree of aspartic acid racemization. However, the protocols for obtaining material for research and workflows related to cartilage processing need to be standardized. The material purification process seems very important because, e.g., in the case of epiglottis cartilage, obtaining elastin for aspartic acid measurements requires a thorough elastin cleansing [43].

Apart from calcification and racemization, the protein glycation level also increases with age, producing advanced glycation end products (AGEs). Their accumulation tints the tissue to a yellow-to-brown tone [46]. Among the tested materials, Achilles tendon, intervertebral disc and costal cartilage, the highest correlation between pigmentation and age was obtained for costal cartilage [47]. The study of the Chinese patients aged 20 to 60 years showed the correlation of $R = 0.861$ and the mean absolute deviation of 3.57 years [48].

Unconfined compression and indentation tests on costal cartilage biomechanical properties in various age groups showed a decrease in Young's Modulus values as the age progressed and no relation to the sex of the deceased [49]. It is also possible to analyze the rib cortical bone matrix changes, as they also correlate with age at death. Among 33 parameters measured by standard bio-mechanical physical and histomorphometry methods, 7 had $R^2 = 0.863$ and a mean absolute error of 4.64 years [50].

In addition to the standard cartilage parameters analyzed, the auricle also should be considered for use in age estimation as it shows a large morphological age-dependent variability. Pre-adults are characterized by a large volume of intercellular substance. Additionally, their auricles are dominated by isogroups of 2–3 chondrocytes, the number of

which decreases with age, while the number of isogroups of 4–5 chondrocytes increases. Moreover, the aggrecan content in cartilage increases with age (1385 arbitrary units for 17–20 years old vs. 2046.3 arbitrary units for 61–75 years old) [51].

Despite many hopes related to the above-presented age-estimation methods using cartilage, these methods are still not standardly used in forensic practice. Forensic and dissection laboratories are not equipped with CT and MRI devices, and their prices are often too high. Likewise, evaluating aspartic acid racemization, cartilage ossification, and protein glycation also requires specialized equipment and skills. Moreover, only a few reports on possible use for postmortem cartilage are available. There are still no standardized protocols for cartilage collection, preparation, and isolation. More extensive research is needed to establish norms for each age range and relate them to inter-individual differences. So far, research has focused on young adults, so cartilage properties in the elderly should also be thoroughly investigated. Furthermore, since the studies using cartilage morphology for age estimation in deceased children and infants are non-existent, this knowledge gap should also be noticed. As the cartilage–bone remodeling of the skeleton happens mainly in childhood, future forensic research should focus on using the knowledge of childhood physiology and prove the usefulness of using cartilage to determine the exact age of a child.

4. Postmortem Interval (PMI) Estimation

Apart from using cartilage in the deceased's age estimation, cartilage is also used to determine the postmortem interval (PMI)—the period that has passed since death. Cartilage undergoes morphological and structural changes from the moment of death, along with the disappearance of nuclear material. Light and scanning electron microscopy for postmortem cartilage imaging can help to demonstrate these changes. Scanning electron microscopy can visualize the orthorhombic shapes in cartilage. They are characteristic of a PMI of 3 to 6 weeks [52]. Subsequent studies have shown that the orthorhombic shapes are crystals made of carbon, nitrogen, oxygen, magnesium, and phosphorous. Their appearance on the synovial surface is related to the pH change during cartilage degradation. So far, these processes were described in pigs, but it would be necessary to study this process in human remains [53]. Research on rabbit auricle cartilage also showed significant modifications in the cartilage matrix density and nuclear material progressing over time. The modifications manifest as changes in cartilage texture and color [54]. Therefore, combining information on cartilage nuclear material and macroscopic and microscopic alterations as PMI progresses may be the key to precise PMI determination.

The advantage of using cartilage for PMI assessment is its low degradation rate compared to other tissues. Li et al. presented a new approach for PMI determination with the Fourier transform infrared spectroscopy [55]. Based on human annular cartilage imaging and using the second derivative transform of 3-point smoothing and extended multiplicative scatter correction method, it was possible to determine PMI with an error of 1.49 days of PMI ranging from 0 to 30 days. Although the method offers high hopes, its improvement is necessary to gain greater accuracy and the possibility of determining PMI beyond 30 days [55]. The method was tested on porcine cartilage previously and showed that some absorbance bands produced by the spectrophotometer correlate significantly with PMI. The study showed that changes in costal cartilage are more pronounced than in rib bones, which further confirms the predominance of cartilage in determining PMI [56]. Similarly, Ramírez et al. showed that Raman spectroscopy and chemometrics could very accurately determine the molecular changes in cartilage during the postmortem period. This method determines PMI by measuring the symmetry of bond vibrations and the orientation of collagen type II, which is correlated with changes in the cartilage viscoelastic and compression properties [57].

Another approach used to determine PMI is chondrocyte viability testing. According to Alibegović et al. [58], the knee joint is the most reliable source of chondrocytes for indirect PMI determination using cell viability assessment. Additionally, they suggested that scanning electron microscopy is more reliable than the standard cell viability analyzer

(CVA). Nonetheless, CVA is a simpler and faster method of PMI determination and, hence, more frequently used and recommended [58]. Standard methods for collagen and hyaline proteoglycan staining appear to be promising new approaches to determining PMI. This method was tested by Alibegović et al. [59] on the 1st, 12th, and 36th-day postmortem with the following staining: Masson's trichrome and Sirius red for collagen, and Alcian blue and Safranin O dyes for proteoglycan. Safranin O without the Fast green was the most reliable for the 36th day postmortem. The authors recommend the method as quick and easy to use and believe that it has the potential to be used in the everyday practice of any forensic laboratory [59].

Animal studies on PMI determination using articular cartilage also add to the knowledge on cartilage properties depending on PMI. Understanding the PMI-dependent changes in the cartilage of different animal species can be a step toward a better judgment of animal crime and poaching lawsuits (primarily related to endangered species). Bolton et al. found out that an aggrecan degradation rate of articular cartilage in a swine model could be used in PMI estimation in cases of exhumation for up to 3 weeks. In this case, the cartilage was analyzed using Western blot against the monoclonal antibody 2-B-6. Since the results were very promising, the authors suggested that the analysis of aggrecan distribution versus PMI should also be tested on human individuals [60].

However, future research should focus on methods determining even longer PMIs. So far, the studies conducted on cartilage samples pertaining to PMIs do not exceed several dozen days. Within this interval, other tissues are often available, and their morphology helps to determine PMI more efficiently by using better-understood methods.

Therefore, in order to start using cartilage in PMI determination in forensic practice, it is necessary to research corpses at least a dozen months old. Moreover, it is imperative to check cartilage properties at various PMI on the human cartilage, as the key studies using cartilage for establishing PMI were only conducted in animals. Furthermore, standardized protocols and norms are needed for each PMI value in order to be able to determine the PMI value as accurately as possible depending on the morphology of the deceased's cartilage.

5. Cause of Death Determination

Due to its location, the costal cartilage is exposed to stab wounds, and the analysis of the surface of both the murder weapon and bone or cartilage can be valuable evidence in criminal cases [61].

In situations where the law enforcement agency is in possession of a potential murder weapon [62], the comparative microscopic analysis of agarose block and costal cartilage fixed in formalin can identify the murder weapon [63]. Such benchmarking may also include cut marks in costal cartilage cast with dental impression material and marks of a suspected knife in cellulose acetate butyrate (a yellow rubbery material) [64]. In addition, a microscopic analysis may be helpful to distinguish fractures due to mechanical compression of the ribcage (e.g., fractures due to resuscitation) from cutting with a knife [65]. Weber et al. [66] describe the case of a homicide of a 27-year-old male victim stabbed multiple times. The microscopic comparison of the stab mark in the third rib with the test mark of the knife in the fifth rib fixed in agarose revealed a sufficient number of matching striations for both marks, leading to the identification of the Swiss Army knife type as the murder weapon [66]. The crucial factor in the microscopic analysis appears to be the time and degree of cartilage hydration. The analysis should be performed immediately after collecting the material during an autopsy. After one week of drying, the cut marks characteristics are almost undetectable, making it extremely challenging to distinguish between cut marks, blunt force fractures, and taphonomic effects [65]. The research by Pounder et al. [67] indicates the possibility of distinguishing knife serration when considering different variants of a possible murder weapon. Although all serrated knives create an "irregularly regular" striated shape in the cartilage, the blades of drop-point knives produce fan-shaped stripes, distinguishing them from straight blades [67]. Pounder and Sim [68] described a trial where a dental cast of the puncture site was used, together with computed tomography

imaging of the punctured cartilage. They appraised CT as a less invasive technique that does not destroy the evidence [68]. However, the blade classification is prone to measurement error. According to Crowder et al. [69], the classification of the blade based on the cartilage fringe pattern is only 79% accurate for serrated and partially serrated blades. The classification accuracy increases to 96% when both blades qualify for one classification (as opposed to straight blades) [69]. An essential aspect of blade identification is the variability in the interpretation of the striation pattern in the cartilage, which may relate to the cartilage calcification degree and the tool insertion angle [70]. Unfortunately, the blade type classification based on the presence of stripes is still characterized by a large, up to 65%, measurement error. It seems necessary to use a classification tree and cross-validation methods and consider the mean interstriation distance to reduce the error rate [71].

Another important aspect is the cartilage histological analysis, which can indicate macroscopically elusive causes of death. Rajs and Thiblin [72] showed that in the case of prolonged death struggle (incomplete hanging, resuscitation, or homicidal neck compression), the histological examination of the fractured superior horns of the thyroid cartilage could show fibrin deposition or leukocytic reaction. Moreover, contraction bands, contraction band necrosis, and 'opaque fibers' can be observed in the adjacent muscle fibers of the thyroid cartilage in the deceased after the high jump and those who died from neck pressure [72]. Future research should focus on detailed postmortem microscopic analysis of the cartilage, which usually does not show any signs of damage in a standard autopsy. An example of such a situation is the case of suicide with the use of a cable tie is described by Langlois and Byard [73]. In this case, no changes in the thyroid cartilage, hyoid bone, or neck muscles were found during postmortem examination despite the persistent cable ties compression of the neck after unconsciousness [73]. Therefore, microscopic analysis of the cartilage may be of key importance in a situation where no injuries are found.

6. Forensic Genetic Analysis

Cartilage is also an excellent material for genetic analyses in personal identification cases [74]. Until now, the gold standard in identifying highly decomposed or exhumed corpses was to extract genetic material from bones and teeth. However, this procedure is time-consuming, expensive, and requires multiple steps, which increases the risk of DNA contamination. Therefore, alternative sources of genetic material, such as eye tissue (cornea and sclera) or cartilage tissue, are being investigated [75]. Importantly, cartilage tissue is free from the chimerism phenomenon that follows bone marrow transplantation, so it seems to be an ideal material for postmortem genetic analysis of unidentified individuals [76].

Cartilage can also be a source of non-degraded DNA after fixation in formalin. STR loci determination in cartilage has comparable success to that obtained from fixed bones and teeth [77]. All newly introduced STR amplification systems for forensic applications showed compatibility with typical biological samples, such as bloodstain, hair, buccal swab, and rib nail, during validation processes, hence, cartilage is often used in individual forensic identification [78]. Cytometric analysis showed a significant correlation between the average DNA content and PMI in human costal cartilage and dental pulp cells stored at different ambient temperatures [79]. Additionally, DNA isolated from cartilage tissue also enables epigenetic analysis. It is possible to establish the hypomethylation and hypermethylation profile of diseases characteristic of cartilage, e.g., temporomandibular joint osteoarthritis [80]. Due to various modulating factors, each tissue has its unique epigenetic pattern [81]. The use of such data for age estimation and genetic identification of people who left their biological traces may be of great importance in reducing the group of suspects [82]. Determining such a pattern in the costal cartilage could narrow the circle of wanted people (missing from a given area) and help select an appropriate comparative material for STR profiling [83]. However, the best cartilage source for forensic genetic testing is yet to be determined. Becker et al. examined 30 intervertebral discs and showed that this source of cartilage provides good quality DNA, characterized by a low degradation rate,

and in sufficient amounts to effectively obtain a complete genetic profile of the deceased by STR-based amplification [84]. Trindade-Filho et al. [85], investigating 21 victims of an air accident in the Amazon rainforest, compared skeletal muscle and hyaline cartilage in the context of DNA to identify decaying individuals. They found that more cartilage samples were suitable for a complete genetic profile evaluation than skeletal muscle samples ($p < 0.01$ for the Wilcoxon signed ranks test) [85].

Genetic phenotyping using next-generation sequencing (NGS) technology seems to be another interesting aspect of DNA isolation [86]. So far, DNA isolated from human remains from World War II allowed for determination of the eye and hair color in 100% of the studied cases [87]. Furthermore, cartilage tissue was used in phenotyping, attempting to reconstruct the deceased's face using alar cartilage dimensions, among other things [88]. Finally, DNA isolation from costal cartilage is faster and cheaper than from bones [89]. Although there is no such research, based on the above, it may be hypothesized that DNA isolated from costal cartilage should also be fit for genetic phenotyping using NGS technology.

Additionally, researchers should focus on using other modern molecular and genetic methods. The available knowledge on miRNAs associated with various diseases seems promising in the context of future cartilage research. According to Shorter et al., osteoarthritis, directly affecting cartilage, is associated with the presence of PPIB, ASS1, LHDB, TPI1, and ARPC4-TTL3 genes [90]. Detecting the presence of these genes when identifying the deceased may narrow the target group to those known to have suffered from osteoarthritis during their lifetime. Of course, in some situations, especially with a very long PMI and favorable conditions for corpse decomposition, isolating the genetic material from cartilage may prove impossible due to its degradation. This is a significant disadvantage of cartilage compared to bone material, which is characterized by a slower degradation rate.

7. Forensic Toxicology

As mentioned earlier, cartilage seems to be an extremely promising source of research material in forensic diagnostics due to its slow decomposition rate. Therefore, it is analyzed for its other possible applications in forensic toxicology. This is particularly important in the case of late-found individuals in which the muscle tissues have decomposed, and the toxicological analysis may facilitate the determination of the cause of death. The studies carried out so far clearly show that it is possible to identify specific substances in the costal cartilage. Moreover, the values of ethanol and isopropanol concentrations in costal cartilage significantly correlate with the concentrations of these substances in blood and urine. Furthermore, the acetone concentration in cartilage correlates with its concentration in urine but not in the blood. These results show that the determinations of some substances in cartilage reliably reflect their concentration in the body at the time of death [91]. However, for some substances, their values in the cartilage exceed their concentration in the blood. A case report by Tomsia et al. [92] presenting suicidal poisoning with sodium nitrite, analyzed its concentration in various tissues and body fluids. The analysis showed that the concentration of nitrite in the blood reached 0.2 µg/mL, while in costal cartilage, it was 3.4 µg/g [92].

It can be clearly stated that postmortem cartilage toxicology is the least studied area of cartilage applications in forensic medicine. New research is needed to ensure unambiguous, quick, and comparable toxicological determinations of substances in cartilage. The proven scientific facts about substances' penetration and deposition rates into cartilage should be used as a basis for future research. The processes related to the deposition of drugs in cartilage are the best known so far. Meier et al. showed that the concentration of ampicillin and sulbactam in cartilage reaches high enough values that exceed the value of the minimum inhibitory concentration (MIC) for biologically important pathogens [93]. Therefore, the deposition of other drugs in the cartilage should be more intensively investigated, especially those that frequently contribute to fatal poisonings.

8. Conclusions

Cartilage is a type of connective tissue that is exceptionally well-tested due to its most prevalent disease—osteoarthritis. However, it seems that despite many studies, the significance of cartilage in forensic medicine is yet underestimated. Thanks to its low metabolism and slow degradation rate, cartilage is a unique material for analysis in cases where it is necessary to determine the deceased's age. It is possible to use a number of its features: ossification rate, aspartic acid racemization, and protein glycation process for age estimation. The same applies to one of the most important issues in forensics—timing the PMI. The literature describes various methods employed to measure PMI, such as chondrocyte viability testing or cartilage extracellular matrix staining. Recent years have also brought much research on postmortem cartilage microscopic analysis, which can be used in criminal cases with a sharp instrument involved. On the other hand, cartilage has proven to be an excellent material for postmortem DNA isolation for the identification of individuals. The role of cartilage in detecting toxic substances being the major or indirect cause of death is the least understood so far. Extending the research on postmortem substances deposition and detection in cartilage is desirable to advance forensic knowledge.

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