ADVANCED IMAGING AND MOLECULAR TECHNOLOGIES IN FORENSIC MEDICINE: NEW DIRECTIONS AND PRACTICAL APPLICATIONS.

Hikmatullaev Ruhulla Zabihullaevich[1].

Ganiyeva Nilufar Xamroevna[2].

Republican Scientific and Practical Center of Forensic Medical

Examination (Tashkent, Uzbekistan)[1].

Tashkent State Medical University, Tashkent Uzbekistan[2].

Abstract: Recent developments in imaging and molecular technologies have significantly reshaped the landscape of forensic medicine. Conventional autopsy, although considered the gold standard for determining cause of death, presents limitations such as invasiveness, time consumption, and cultural or legal restrictions. Modern imaging techniques, including post-mortem computed tomography (PMCT), magnetic resonance imaging (MRI), and three-dimensional reconstruction—collectively referred to as virtual autopsy—offer a non-invasive alternative for comprehensive visualization of internal structures. These methods allow accurate detection of fractures, hemorrhages, air embolism, and pathological changes that may not be evident during traditional dissection.

In parallel, molecular methods have become indispensable tools in forensic investigations. DNA profiling remains the cornerstone for human identification, while novel techniques such as RNA-based analysis, proteomics, and metabolomics are increasingly employed to assess wound vitality, determine the post-mortem interval, and explore biochemical alterations associated with death. The concept of molecular autopsy, which focuses on genetic and epigenetic factors in sudden unexplained deaths, further underscores the evolving role of molecular science in forensic diagnostics.

This review highlights the current applications, advantages, and limitations of modern imaging and molecular approaches in forensic medicine. It also emphasizes the importance of integrated protocols that combine these technologies to enhance diagnostic accuracy, preserve evidence integrity, and ensure ethical standards in medico-legal investigations.

Keywords: forensic imaging, post-mortem CT, MRI, virtual autopsy, molecular autopsy, DNA analysis, forensic medicine.

Introduction: Forensic medicine has long relied on traditional autopsy as the primary method for determining the cause, manner, and circumstances of death. Despite its continued recognition as the gold standard, the conventional autopsy procedure faces inherent challenges. These include its invasive nature, time-consuming protocols, and,

in many instances, limitations imposed by cultural, religious, or legal considerations (Rutty et al., 2017). Moreover, certain categories of injuries and pathological processes—especially those associated with vascular compromise, subtle hemorrhages, or microstructural alterations—often remain under-recognized during conventional autopsy, even when conducted meticulously. For example, early ischemic lesions, diffuse axonal injury, microvascular thrombosis, and occult hemorrhages in deep tissue planes or parenchymal organs can evade gross visual inspection due to their microscopic scale or absence of overt macroscopic changes. Similarly, post-traumatic gas embolism, vascular dissections, or intramural hematomas may present without characteristic surface indicators, making them virtually undetectable through dissection alone. Such limitations increase the risk of incomplete or equivocal diagnostic interpretations, particularly in complex trauma, sudden unexplained deaths, or cases requiring reconstruction of precise injury mechanisms.

Advanced postmortem imaging techniques—such as contrast-enhanced CT angiography, high-resolution MRI, and radiological 3D reconstruction—address these gaps by enabling visualization of vascular patency, microhemorrhages, and spatial relationships among lesions without destructive sampling. When combined with molecular analyses (e.g., immunohistochemistry, proteomics, and mRNA degradation profiling), these tools provide a multidimensional perspective that augments both morphological and functional interpretation. This integration not only enhances diagnostic completeness but also strengthens the forensic validity of conclusions in legal and investigative contexts (Jackowski et al., 2018).

The past two decades have seen substantial advancements in imaging technologies, which have significantly transformed forensic investigations. Techniques such as post-mortem computed tomography (PMCT), magnetic resonance imaging (MRI), and three-dimensional reconstruction—collectively termed "virtual autopsy" or "virtopsy"—offer non-invasive visualization of internal anatomy with high spatial resolution. PMCT has proven highly effective in detecting skeletal fractures, gas embolisms, and hidden hemorrhages, while MRI excels in identifying soft tissue and brain injuries that might be overlooked during conventional autopsy (Thali et al., 2019; Ampanozi et al., 2020). These methods also facilitate secure digital archiving of high-fidelity data, including three-dimensional reconstructions, volumetric imaging datasets, and metadata, which can be preserved indefinitely without degradation. Such digital repositories enable independent re-examination, second-opinion consultations, and retrospective case reviews, even years after the original investigation, without the need for repeated invasive procedures on the body. This capability significantly enhances transparency by providing auditable, tamper-resistant records and supports reproducibility through standardized imaging protocols and objective datasets. Furthermore, digital integration allows interoperability with machine-learning pipelines for automated feature extraction, cross-case comparisons, and population-

level analytics. Collectively, these advantages strengthen the scientific rigor and legal defensibility of forensic investigations, ensuring that conclusions remain verifiable and open to peer scrutiny in both judicial and academic contexts (Morgan et al., 2021).

Simultaneously, molecular techniques have revolutionized the forensic sciences by providing precise tools for biological identification and functional analysis. DNA profiling remains indispensable for individual identification in criminal cases and disaster victim recovery, supported by short tandem repeat (STR) analysis and mitochondrial DNA sequencing (Kayser, 2017). More recently, molecular methods have expanded beyond identity confirmation. RNA-based markers assist in estimating wound vitality, while proteomics and metabolomics enable the study of post-mortem biochemical changes, improving time-of-death estimation (Madea & Saukko, 2020). Furthermore, the concept of molecular autopsy—using next-generation sequencing to investigate genetic variants linked to sudden unexplained deaths—has gained significant clinical and forensic relevance (Bagnall et al., 2016).

The integration of imaging and molecular approaches provides a synergistic framework that enhances the reliability of forensic investigations. This combined methodology not only improves diagnostic precision but also addresses ethical concerns by reducing invasiveness and enabling virtual analysis in cases where autopsy is restricted (Thali et al., 2019). Despite their advantages, these technologies present challenges, including high implementation costs, the need for specialized training, and legal considerations regarding data confidentiality (Morgan et al., 2021).

This review aims to present an updated overview of modern imaging and molecular methods in forensic medicine, discuss their current applications, and evaluate the opportunities and limitations associated with their integration into routine forensic practice.

Relevance: The evolution of forensic medicine in the 21st century has been strongly influenced by technological innovations aimed at improving diagnostic accuracy and addressing the limitations of conventional autopsy. While traditional autopsy remains the cornerstone of medico-legal investigations, it presents several challenges, including invasiveness, cultural and religious objections, and difficulties in detecting certain pathological conditions such as gas embolism or subtle fractures (Rutty et al., 2017). Furthermore, contemporary forensic practice requires methods capable of preserving evidence integrity, ensuring reproducibility, and meeting the demands for transparency in judicial processes (Ampanozi et al., 2020).

Modern imaging techniques, such as post-mortem computed tomography (PMCT), magnetic resonance imaging (MRI), and virtual autopsy, provide non-invasive visualization of internal anatomy, enabling rapid and detailed assessment of injuries without disrupting the body (Thali et al., 2019). These methods have become indispensable in mass disasters, aviation accidents, and cases involving complex trauma, where conventional autopsy alone may be insufficient (Morgan et al., 2021).

Simultaneously, the increasing role of molecular methods in forensic medicine reflects the need for precision and objectivity. DNA profiling, RNA-based analysis, and proteomic studies contribute to identity verification, estimation of wound vitality, and determination of the post-mortem interval (Kayser, 2017; Madea & Saukko, 2020). The combined use of imaging and molecular technologies has therefore become essential for establishing reliable and scientifically sound medico-legal conclusions (Bagnall et al., 2016).

This integration aligns with global trends toward minimally invasive, ethically compliant, and technologically advanced forensic approaches, ensuring that medico-legal investigations meet both scientific and societal expectations.

Materials and Methods: This review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines to ensure methodological transparency and reproducibility (Moher et al., 2009). A comprehensive literature search was carried out in electronic databases, including PubMed, Scopus, Web of Science, and Google Scholar, covering publications from January 2010 to December 2024. The keywords and MeSH terms applied were: forensic imaging, post-mortem computed tomography (PMCT), magnetic resonance imaging (MRI), virtual autopsy, molecular autopsy, DNA analysis, RNA-based forensic markers, and proteomics in forensic medicine. Boolean operators AND/OR were used to combine terms and refine search results (Higgins et al., 2019).

Inclusion criteria were:

• Peer-reviewed articles in English.

• Studies addressing imaging and/or molecular methods applied in forensic medicine.

• Original research, systematic reviews, and meta-analyses published within the last 15 years.

Exclusion criteria were:

• Non-peer-reviewed sources (e.g., conference abstracts without full text).

• Studies unrelated to forensic applications (e.g., clinical imaging or genetics without forensic context).

After removing duplicates, titles and abstracts were screened for relevance, followed by full-text assessment. Data extraction focused on the following domains: imaging modality (PMCT, MRI, virtopsy, angiography), molecular technique (DNA, RNA, proteomics, metabolomics), primary forensic application, advantages, limitations, and reported diagnostic accuracy (Madea & Saukko, 2020; Rutty et al., 2017).

The final selection included **68 studies** that met the eligibility criteria. The methodological quality of included studies was evaluated using the QUADAS-2 tool for diagnostic accuracy studies (Whiting et al., 2011).

Results: The integration of advanced imaging and molecular diagnostic methods into forensic practice significantly enhanced the precision and comprehensiveness of post-mortem examinations. **Post-mortem computed tomography (PMCT)** demonstrated outstanding diagnostic capabilities in detecting skeletal injuries, occult fractures, and concealed hemorrhages without invasive intervention, often surpassing the traditional autopsy in terms of fracture visualization and detection of gas embolism (Ross et al., 2020). In trauma-related fatalities, PMCT was particularly effective in identifying pneumothorax, internal bleeding, and organ displacement, making it a valuable adjunct in forensic pathology (Roberts et al., 2018). **Magnetic resonance imaging (MRI)**, on the other hand, provided superior evaluation of soft tissue injuries, intracranial pathology, and cardiac anomalies, which are often challenging to detect using conventional techniques (Jackowski et al., 2018).

At the molecular level, DNA profiling continues to represent the gold standard for human identification in forensic science due to its high discriminatory power, stability, and well-established evidentiary framework. However, its utility is primarily limited to individualization and kinship analysis; it offers little direct information on the timing or vitality of injuries. To address this gap, research has shifted toward the exploration of dynamic molecular biomarkers capable of reflecting biological processes associated with trauma and death.

Among these, messenger RNA (mRNA) and microRNA (miRNA) have emerged as particularly promising tools. Unlike genomic DNA, which remains relatively constant throughout life, RNA species are highly responsive to physiological stress, cellular damage, and post-mortem degradation, making them informative indicators of both antemortem and postmortem events. For instance, the differential expression of mRNA transcripts involved in inflammation, coagulation, and hypoxia pathways can provide insights into wound vitality, helping to distinguish injuries sustained during life from those inflicted postmortem. Similarly, miRNAs—owing to their remarkable stability in body fluids and tissues—offer additional advantages for estimating the post-mortem interval (PMI) and characterizing tissue-specific responses to trauma.

Recent studies have demonstrated that certain mRNA and miRNA signatures exhibit predictable degradation kinetics correlated with time since death, enabling the development of molecular PMI estimation models that complement traditional morphological and physicochemical methods. Moreover, these biomarkers can be analyzed using quantitative PCR, next-generation sequencing, or microarray platforms, facilitating multiplex approaches that integrate with proteomic and metabolomic profiling for a comprehensive molecular autopsy.

The integration of such biomarkers into forensic workflows represents a paradigm shift, moving beyond static structural observations toward dynamic molecular interpretations of injury and death processes. These innovations not only enhance diagnostic precision but also contribute to more robust, scientifically validated

reconstructions of the circumstances surrounding death, thereby strengthening the evidentiary value of forensic conclusions (Madea & Saukko, 2019).

Proteomic and metabolomic profiling has further enabled the detection of biochemical shifts linked to hypoxia, ischemia, and systemic inflammatory responses, offering crucial insights in cases where morphological evidence alone was insufficient (Musshoff et al., 2021). In addition, **molecular autopsy**—based on next-generation sequencing—allowed the identification of genetic mutations underlying sudden cardiac deaths in young individuals, thereby supporting both forensic investigations and preventive measures for relatives (Bagnall et al., 2016).

The combined use of imaging and molecular methods proved particularly valuable in cases with **religious or cultural restrictions** on full autopsy, providing minimally invasive yet comprehensive forensic evaluations (Rutty et al., 2013). Integration of radiological findings with genetic and biochemical data has revolutionized the diagnostic process in forensic medicine, offering a more comprehensive and scientifically grounded approach to determining both the cause and manner of death. Radiological imaging techniques, such as computed tomography (CT) and magnetic resonance imaging (MRI), provide detailed visualization of internal anatomical structures, enabling the detection of injuries, fractures, and internal hemorrhages that may not be visible during external examination. When combined with genetic analysis, particularly DNA profiling, and advanced biochemical testing, this approach enhances diagnostic precision by addressing both structural and molecular evidence.

For instance, radiological imaging can identify skeletal or soft tissue injuries, while molecular methods can confirm biological relationships, detect genetic predispositions, or reveal signs of metabolic or toxicological alterations. The integration of these data sets facilitates differentiation between antemortem and postmortem injuries, helps in identifying subtle pathological changes, and supports reconstruction of traumatic events with greater accuracy.

This multidimensional strategy minimizes the risk of diagnostic errors that can arise from relying on a single method and improves the evidentiary value of forensic conclusions in legal proceedings. Consequently, the adoption of radiological, genetic, and biochemical integration as a standard protocol is not merely advantageous but essential for modern forensic practice, ensuring a scientifically validated and reproducible approach to death investigation (Grabherr et al., 2018).

Conclusion: The integration of modern imaging techniques and molecular methods into forensic medicine has significantly improved the accuracy, reliability, and efficiency of forensic investigations. Advanced imaging modalities, such as postmortem computed tomography (PMCT) and magnetic resonance imaging (MRI), enable detailed visualization of internal injuries, bone fractures, and vascular pathologies without the need for invasive autopsy procedures, offering a complementary tool in both routine and complex forensic cases (Thali et al., 2013).

These imaging technologies are especially useful in detecting subtle or concealed injuries, assisting in determining cause and manner of death in cases involving trauma, drowning, or sharp-force injuries (Rutty et al., 2017).

Similarly, molecular methods have transformed forensic diagnostics by providing high-resolution insights at the cellular and genetic level. Techniques such as DNA profiling, RNA-based analysis, and proteomics allow for the identification of individuals, estimation of the postmortem interval, and evaluation of tissue vitality in traumatic injuries (Vidaki & Kayser, 2018). The application of microRNA markers and advanced omics-based approaches enables differentiation between ante-mortem and post-mortem injuries and contributes to solving cases involving sexual violence, mass disasters, and highly degraded samples (Fridman et al., 2020).

The combined application of imaging and molecular technologies establishes a multidisciplinary paradigm, ensuring that forensic medicine meets the demands of modern judicial systems for scientifically robust, minimally invasive, and ethically sound investigations (Morgan et al., 2020). Despite these advances, challenges remain regarding cost-effectiveness, accessibility in low-resource settings, and the need for standardized protocols to ensure global reproducibility and legal acceptance of these methods (Christensen et al., 2014). Future directions should focus on integrating artificial intelligence and machine learning for automated image interpretation, expanding molecular biomarkers for forensic use, and creating universal guidelines to harmonize practices across jurisdictions (Dedouit et al., 2021).

Thus, modern imaging and molecular approaches represent a pivotal step-change in forensic medicine, moving the field from predominantly descriptive assessments to quantitatively robust, multimodal inference. High-resolution post-mortem CT and MRI, 3D surface scanning, and image-guided sampling enable objective documentation, reconstruction of injury mechanisms, and discrimination between antemortem and postmortem changes, while preserving bodies for re-examination. In parallel, next-generation sequencing, DNA phenotyping, epigenetic age and tissue-origin markers, proteomics/metabolomics, microbiome profiling, and "molecular autopsy" in unexplained deaths expand the detectable signal beyond gross and histologic findings. When these streams are integrated with validated pipelines, quality assurance, and probabilistic interpretation (e.g., likelihood ratios), they strengthen the evidentiary value of forensic conclusions by increasing sensitivity and specificity, improving reproducibility, and making uncertainty explicit. This trajectory—from the foundational advent of DNA fingerprinting to comprehensive post-mortem multi-omics—has systematically raised the scientific bar for identification, cause- and manner-of-death opinions, and injury interpretation (Jeffreys et al., 1985; Banaschak et al., 2020).

Crucially, these technologies support justice by enhancing transparency (audit trails and revisitable data), reducing observer bias through standardized protocols and

automated analytics, and meeting admissibility expectations through validation and inter-laboratory concordance. They also promote minimally invasive workflows that respect cultural and religious considerations, facilitate rapid disaster victim identification, and enable targeted resource use in overloaded systems. Ongoing challenges—costs, training, interoperability, and ethical governance of sensitive genetic data—are active fronts of development, but the direction of travel is clear: scientifically validated imaging and molecular methods now form the backbone of a modern, reliable, and courtroom-ready forensic practice (Jeffreys et al., 1985; Banaschak et al., 2020).

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