Legal Medicine 82 (2026) 102820

Contents lists available at ScienceDirect

Legal Medicine

journal homepage: www.elsevier.com/locate/legalmed

Fibronectin expression as a marker of hypoxia in asphyxial deaths: A pilot study☆

Tommaso D'Anna [a,b,c], Rosario Barranco [d,e], Simona Merighi [b], Valeria Cancila [b], Ginevra Malta [f], Stefania Zerbo [c,f], Giuseppe Davide Albano [c,f], Giuseppe Di Pietrantonio [d], Isabella Caristo [d], Francesco Ventura [d,e], Antonina Argo [c,f,1], Beatrice Belmonte [b,*,1]

[a] Department of Biomedicine and Prevention, Tor Vergata University of Rome, Rome, Italy
[b] Tumor Immunology Unit, Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties, University of Palermo, Palermo, Italy
[c] Legal Medicine Unit, Policlinic University Hospital "Paolo Giaccone", Via del Vespro 129, 90138 Palermo, Italy
[d] Department of Legal and Forensic Medicine, Health Science Department (DISSAL), University of Genoa, Via De Toni, 12, 16132 Genoa, Italy
[e] I.R.C.C.S. Ospedale Policlinico San Martino Teaching Hospital, Largo Rosanna Benzi, 10, 16132 Genoa, Italy
[f] Section of Legal Medicine, Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties, University of Palermo, Palermo, Italy

ARTICLE INFO

Keywords:
Asphyxial deaths
Hypoxia biomarkers
Fibronectin
Immunohistochemistry
Forensic pathology

ABSTRACT

Aim: Asphyxial deaths represent a major diagnostic challenge in forensic practice, as classical morphological signs are nonspecific and may overlap with other causes of sudden death. Fibronectin (FN), an extracellular matrix glycoprotein sensitive to hypoxia, is emerging as a candidate biomarker of oxygen deprivation. We investigated its expression in cardiac tissue from asphyxial deaths compared with traumatic controls.

Methods: We retrospectively collected formalin-fixed, paraffin-embedded left ventricular myocardium from autopsies in Palermo and Genoa (2021–2025). The cohort included 54 cases: 45 asphyxial deaths (5 smothering, 4 strangulation, 15 hanging, 17 drowning, 4 chemical asphyxia) and 9 non-asphyxial traumatic controls. We performed FN immunohistochemistry (IHC) with a monoclonal antibody and quantified FN-positive cardiomyocytes using dedicated image-analysis software.

Results: We found significantly higher FN expression in all asphyxial deaths compared with controls (mean 11.7 ± 3.6%). The highest intensities were observed in smothering (46.1 ± 7.2%) and strangulation (45.0 ± 8%), followed by chemical asphyxia (35.1 ± 9.8%), hanging (27.8 ± 6.6%), and drowning (21.4 ± 5.9%). FN levels varied between subgroups, but their consistent elevation confirms its reliability as a marker of hypoxic injury in asphyxia.

Conclusion: FN upregulation in the myocardium is a consistent hallmark of asphyxial deaths, reflecting extracellular matrix changes under hypoxia. FN identifies hypoxic stress rather than mechanism-specific asphyxia; nonetheless, it can be a valuable aid in forensic diagnosis, especially when morphology alone is inconclusive. Integration of FN IHC with conventional autopsy and histology could enhance diagnostic accuracy and support medicolegal assessments of asphyxial death.

1. Introduction

Asphyxia refers to the deprivation of oxygen with concurrent accumulation of carbon dioxide, leading to hypoxemic-hypercapnic injury and, when prolonged, to irreversible death. Mechanisms are heterogeneous and include mechanical airway obstruction (e.g., hanging, smothering, or choking), impairment of gas exchange due to drowning or chemical agents, and suppression of the respiratory centre or

Abbreviations: AQP, aquaporin; ECM, extracellular matrix; EDG, extra domain B; FN, fibronectin; FFPE, formalin-fixed, paraffin-embedded; FTIR, Fourier-transform infrared; IHC, immunohistochemistry; PBS, phosphate-buffered saline; PMI, postmortem interval; SCD, sudden cardiac death.

☆ This article is part of a special issue entitled: 'Proceedings Congress Forensic Science' published in Legal Medicine.

* Corresponding author at: Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties (PROMISE) – University of Palermo, Corso Tukory 211, 90134 Palermo, Italy.
E-mail address: beatrice.belmonte@unipa.it (B. Belmonte).

[1] Beatrice Belmonte and Antonina Argo contributed equally as co-last authors.

https://doi.org/10.1016/j.legalmed.2026.102820
Received 7 September 2025; Received in revised form 4 February 2026; Accepted 22 February 2026
Available online 23 February 2026

neuromuscular function. Regardless of aetiology, asphyxial deaths share a standard pathophysiological sequence. Early loss of oxygen delivery triggers anaerobic metabolism with conversion to lactate and rapid metabolic acidosis. Hypercapnia develops because carbon dioxide elimination is impaired; this leads to cerebral vasodilation and increased intracranial pressure, promoting hypoxic-ischaemic encephalopathy and brainstem dysfunction. Because external signs like petechiae, cyanosis, and congestion are nonspecific, postmortem diagnosis is complex, and forensic pathologists now turn increasingly to biochemical and immunohistochemical markers to detect hypoxic injury [1].

Fibronectin (FN) is a high-molecular-weight glycoprotein of the extracellular matrix (ECM) composed of two similar subunits containing binding domains for collagen, integrins, heparin, and fibrin [2]. It exists as soluble plasma and insoluble cellular FN. In canonical functions, FN mediates cell adhesion and migration by binding to integrin receptors and other ECM proteins, supports mesodermal differentiation and proliferation, and organizes the provisional fibrin-FN matrix that guides fibroblast and endothelial ingrowth during wound healing [2,3]. FN fibrils capture growth factors, limiting their diffusion and concentrating cytokines and morphogens, which in turn shape gene expression and tissue morphogenesis [3,4]. FN also mediates mechanotransduction [4].

In hypoxic conditions, FN exhibits several non-canonical roles that actively modulate ECM remodeling, cell adhesion, and inflammatory pathways. Since the diagnosis of asphyxia in forensic pathology remains notoriously challenging, the identification of molecular signatures that mirror hypoxia-driven tissue changes is crucial. In this perspective, FN emerges as a particularly promising biomarker, as suggested by its potential utility as an auxiliary marker in asphyxial deaths, where the poor specificity of gross findings highlights the value of integrating histopathology with immunohistochemistry targeting FN and related markers. On this rationale, we designed the present study.

2. Materials and methods

2.1. Human tissue samples

Formalin-fixed, paraffin-embedded (FFPE) myocardial samples were enrolled from the Institutes of Legal Medicine of the Universities of Palermo and Genoa. The study cohort comprised cases of asphyxial deaths 45 and 9 non-asphyxial traumatic controls, classified as follows: smothering ($n = 5$), strangulation ($n = 4$), hanging ($n = 15$), drowning ($n = 17$), and chemical asphyxia ($n = 4$). For each case, only one sample was collected, specifically from the posterior wall of the left ventricular myocardium.

Exclusion criteria included visible signs of putrefaction, a postmortem interval (PMI) exceeding 96 h, cardiac trauma, known cardiac disease, moderate to severe coronary artery disease, previous myocardial infarction, and age above 65 years. As a reference group, we included control cases ($n = 9$) consistent with traumatic deaths.

2.2. IHC and quantitative immunolocalization analyses

Serial sections (3 μm thick) were cut from FFPE blocks, deparaffinized, rehydrated, and subjected to antigen retrieval using Novocastra Epitope Retrieval Solution (pH 9; Leica Biosystems) in a thermostatic bath at 98.5 °C for 30 min. Sections were then cooled to room temperature and rinsed in phosphate-buffered saline (PBS). Endogenous peroxidase activity was quenched with 3% H_2O_2, followed by Fc blocking with 0.4% casein in PBS (Leica Biosystems).

Tissue sections were incubated with rabbit monoclonal FN (clone F14, 1:2 pH9; AN569-5 M, BioGenex). Signal detection was carried out using the Novolink Polymer Detection System (Leica Biosystems) with DAB (3,3′-diaminobenzidine, Novocastra) as the substrate chromogen. Nuclei were counterstained with Mayer's hematoxylin.

2.3. Image acquisition and quantitative analyses

We analyzed and imaged slides under a Zeiss Axioscope-A1, and micrographs were collected using a Zeiss Axiocam 503 Color digital camera with the Zen 2.0 Software (Zeiss).

We performed quantitative analyses by calculating the average percentage of positive signals in five randomly selected, nonoverlapping fields at medium-power magnification (×200). Images were acquired in a blinded fashion using Positive Pixel Count v9 (2 + moderate positivity: signal intensity range 175–100; 3 + strong positivity: signal intensity range 100–0) ImageScope software (Aperio ImageScope version 12.3.2.8013, Leica Biosystems).

2.4. Institutional review board, informed consent and data availability statements

The study was conducted in accordance with the Declaration of Helsinki. Ethical approval and informed consent were not required, as only archival, anonymized FFPE samples from judicial autopsies were analyzed, in compliance with national forensic regulations.

We did not require specific informed consent for this study because we analyzed anonymized tissue samples obtained from judicial autopsies. National regulations allow the use of such material for scientific purposes without consent from next of kin, provided that we do not use personally identifiable information.

The data supporting the findings of this study are not publicly available due to ethical and privacy restrictions, as they involve patient-related clinical and histopathological information. We may provide access to anonymized data upon reasonable request.

3. Results

The investigation of FN expression across our cohort unfolded in progressive steps, encompassing a semiquantitative evaluation, followed by the analysis of quantification of signal, and a structured quantitative confirmation. We first applied IHC to control kidney FFPE sections, where tubular cells of the cortical region displayed a pattern of immunoreactivity, with a moderate density of positive cells with cytoplasmic label. This step validated the staining protocol and provided a reliable internal benchmark for subsequent analyses.

To ensure reproducibility, two operators (B.B. and V.C.) independently performed a semiquantitative evaluation of FN expression on all samples, noting a discernible increase in signal intensity within the asphyxial death group compared to controls. Subsequently, B.B. randomly selected five areas at × 200 magnification to perform signal quantification using the dedicated image-analysis tool, designed for objective signal quantification. This dual-level approach confirmed the preliminary observation: FN expression was consistently upregulated in asphyxial deaths compared with controls, with a heterogeneous distribution among the different subtypes.

At the quantification analysis, we observed a marked and significant upregulation of FN expression in all cases of asphyxial death relative to the control group.

Importantly, despite variation in group sample sizes, FN expression did not follow a uniform pattern: the highest intensity was present in deaths due to smothering and strangulation, where staining intensity and percentage of staining reached their peak.

By contrast, hanging, drowning, and chemical asphyxia showed comparatively lower – though still significantly elevated – expression levels, underscoring the variability of FN deposition across distinct mechanisms of asphyxial death.

In quantitative terms, the control group exhibited a mean percentage of FN signal of 11.7 ± 3.6%, whereas asphyxial deaths demonstrated markedly higher values: smothering, 46.1 ± 7.2% ($n = 5$); strangulation, 45.0 ± 8% ($n = 4$); hanging, 27.8 ± 6.6% ($n = 15$); drowning, 21.4 ± 5.9% ($n = 17$); chemical asphyxia, 35.1 ± 9.8% ($n = 4$) (Fig. 1).

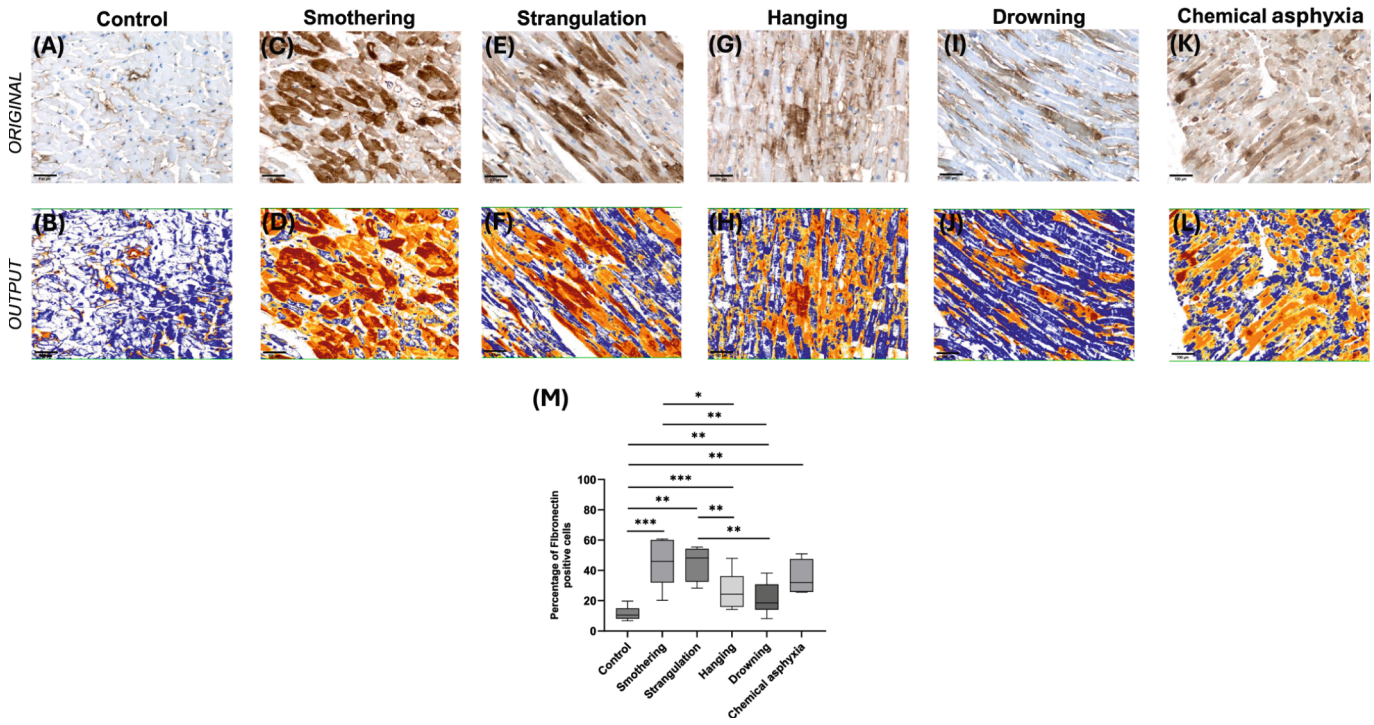

Fig. 1. Cardiomyocytes expressing FN increase in the asphyxial death group. (A)-(L), representative input (up) and output (down) images and quantitative analyses of FN: (A) and (B) control group, (C) and (D) smothering group, (E) and (F) strangulation group, (G) and (H) hanging group, (I) and (J) drowing group, and (K) and (L) chemical asphyxia group. Original magnification × 200. Scale bars 100 μm. (M) Statistical analysis: two-tailed unpaired Student *t* test. Mean ± standard error shown; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

This variability suggests that, while FN upregulation represents a shared hallmark of asphyxial deaths, the magnitude of expression may reflect differences in the pathophysiological cascade triggered by each asphyxial mechanism.

4. Discussion

This study shows upregulation of FN in cardiac tissue from asphyxial deaths compared with controls. This consistent finding across semi-quantitative and quantitative analyses suggests that FN can mark tissue hypoxia and reflect myocardial remodeling under severe oxygen deprivation.

FN displays several non-canonical roles in hypoxic conditions, undergoing a functional shift driven by an oxygen-sensitive rewiring of the integrin-TGF-β-mechanotransduction axis that usually governs fibrillogenesis. In canonical settings, FN polymerization into load-bearing fibrils requires α5β1-integrin engagement, cytoskeletal tension to unfold type III repeats and expose cryptic self-assembly sites, and a TGF-β feed-forward loop that augments contractility and matrix assembly. Hypoxia, however, uncouples this circuit: hypoxia increases FN transcription and secretion but blunts the mechanical program needed for fibrillogenesis, causing FN to accumulate pericellularly in non-fibrillar or loosely organized forms. Studies show this "secretion-without-assembly" phenotype during epithelial transdifferentiation under low O_2, in line with the force-dependent, multi-stage integrin/TGF-β control of FN assembly. Overall, hypoxia shifts FN from a structural scaffold to a dynamic organizer of biochemical signaling, a clearly non-canonical role [5,6].

Within hypoxic microenvironments – exemplified by tumors but relevant to any severely hypoxemic tissue – FN acts as a signaling hub that coordinates integrin crosstalk, growth-factor bioavailability, and stromal activation. Comprehensive reviews show that hypoxia conditions the stromal compartment (notably cancer-associated fibroblasts) to secrete and assemble FN-rich provisional matrices, alter the integrin repertoire (α5β1 and αv-class), and potentiate FAK/Src and YAP/TAZ mechanotransduction. In this configuration, pericellular and partially polymerized FN presents and concentrates cytokines and growth factors (e.g., latent TGF-β via LTBP-1; VEGF at Hep II sites), thereby amplifying angiogenesis, leukocyte trafficking, and invasive cell behavior – features that are archetypally non-canonical for a matrix glycoprotein [7,8].

Hypoxia's influence is not limited to fibrillogenesis blockade; it also reprograms downstream pathway usage. In non-small-cell lung cancer, substrate-bound FN activates FAK/MAPK signaling, elevates HIF-1α, and upregulates WISP3, thereby sustaining angiogenesis, epithelial-mesenchymal transition (EMT), and stemness in vitro and in vivo – an FN-driven, HIF-linked circuit in which signaling clearly outweighs architecture. This same axis feeds into Wnt activation, further entrenching a pro-angiogenic, pro-invasive state [9].

Splice-variant usage deepens this non-canonical turn. Hypoxic, remodeling microenvironments strongly upregulate the extra domain B (EDB) isoform (FN-EDB), which endows FN with distinct receptor-ligand interactions (notably with αvβ3/αvβ5) and signaling behaviors. FN-EDB not only enhances tumor cell adhesion and motility via FAK/PI3K/AKT and ERK/MAPK cascades, but also interfaces with atypical β1-integrin signaling from endosomal pools, sustaining intracellular FAK activity away from the plasma membrane and promoting invasion, anoikis resistance, and EMT. FN-EDB promotes immune evasion within the tumor microenvironment and enhances angiogenesis by activating endothelial integrins and HIF-1-dependent pathways, including VEGF, DLL4/Notch, and MMP remodeling. In hypoxic settings, FN shifts these functions to a regulator of signaling, vascular remodeling, and immune responses [10].

Mechanistically, hypoxia is synergistic with TGF-β in specific tissues, further steering FN toward signaling roles. In skeletal muscle, oxygen deprivation cooperates with TGF-β to upregulate CCN2/CTGF specifically in myofibers (not fibroblasts) through an HIF-1-dependent but Smad3-independent route, with ERK/JNK and SP1/SP3 as critical intermediates. CCN2/CTGF is a matricellular amplifier that remodels ECM

and augments integrin signaling, thereby biasing the microenvironment toward fibrosis and paracrine activation rather than simple matrix replacement. This concept illustrates that hypoxia redirects ECM components – including FN – toward pro-remodeling and pro-signaling functions [11].

Importantly, not all regulation of FN in these settings is HIF-driven. The VHL tumor-suppressor pathway controls proper FN deposition independently of hypoxia; VHL competence promotes FN expression and matrix incorporation, whereas its loss disrupts deposition. This finding underscores that oxygen tension is only one determinant of FN's structural fate; when VHL/ECM surveillance or the mechanical limb of the integrin circuit is perturbed, FN remains non-fibrillar and increasingly "signaling-prone" [12].

Crosstalk with endocrine cues can also gate hypoxia-like FN programs. Triiodothyronine (T3) increases FN expression through HIF-1 activation – demonstrating that the "hypoxic" FN transcriptional module can be engaged by non-hypoxic stimuli, broadening the circumstances in which FN adopts non-canonical roles. Such endocrine-HIF intersections help explain FN upregulation in heterogeneous clinical contexts beyond frank ischemia [13,14].

From a systems perspective, current syntheses of hypoxia signaling emphasize its integration with PI3K-mTOR, ERK/MAPK, NF-κB, and ER-stress axes. FN sits at the convergence of these pathways: as an integrin ligand, it controls FAK/Src and RhoA/ROCK mechanics; as a growth-factor reservoir, it sculpts cytokine gradients; and as a splicing-variant platform (EDB), it re-specifies receptor usage and endosomal signaling. The net effect in low-O_2 milieus is an FN-centric network that favors inflammatory amplification, angiogenesis, and fibro-activation – precisely the non-canonical functions observed in hypoxic disease states [15].

In sum, hypoxia transforms FN from a structural macromolecule into a microenvironmental signal integrator. By elevating secretion while limiting fibrillogenesis, by reprogramming integrin and splice-variant engagement (notably FN-EDB), and by coupling to HIF-conditioned TGF-β, MAPK, and Wnt circuits (e.g., WISP3 induction), FN becomes a platform that orchestrates early stress responses, vascular remodeling, and immune modulation. This framework accounts for the abundant yet poorly fibrillar FN in hypoxic tissues. It clarifies why, in hypoxemic settings – including those encountered in asphyxial pathology – FN predominantly exerts non-canonical roles centered on signaling, angiogenesis, and stromal re-patterning rather than on mature fibrillar load-bearing [5,6,9,11,15].

Fig. 2 below illustrates the shift of FN from a structural ECM component to a signaling platform under the influence of hypoxia and regulatory pathways such as VHL loss.

Experimental models of acute myocardial infarction demonstrate irregular accumulation of FN in the cytoplasm and interstitium of cardiomyocytes; quantitative immunohistochemistry (IHC) shows that the area of FN-positivity in early infarction is approximately 75 times greater than in mechanical asphyxia and control cases [16]. Upregulated FN in hypoxic myocardium is part of a provisional ECM; its degradation products act as chemotactic signals for neutrophils and macrophages and modulate inflammatory recruitment. Moreover, FN fibrils can bind more than forty growth factors and cytokines, including TGF-β and platelet-derived growth factor, altering their availability during hypoxic injury [4]. These properties suggest that increased FN deposition may indicate early hypoxic stress before overt necrosis. Conversely, studies on drowning show no significant difference in FN expression between saltwater, freshwater drowning, and controls [17], and analyses of ligature marks report strong FN positivity in dermal vessels but emphasize that its reliability as a vitality marker is controversial [18].

ECM dynamics intertwine with the pathophysiology of asphyxial deaths. During mechanical obstruction or immersion, hypoxia and reperfusion induce reactive oxygen species, activate matrix metalloproteinases, and disrupt cellular adhesion. Early deposition of FN within the myocardium and vascular walls may represent an adaptive attempt at stabilizing the ECM, recruiting inflammatory cells, and sequestering growth factors [1,2,4,5,7,9,10,15,16].

FN is a multifunctional ECM glycoprotein involved in cell adhesion, wound healing, and tissue repair, whose expression is known to be modulated by hypoxic stress and inflammatory stimuli [19,20].

In line with previous studies that have demonstrated hypoxia-driven upregulation of FN in ischemic myocardium and other tissues [21,22], our data suggest that asphyxial deaths, irrespective of their specific etiology, converge on a typical molecular response characterized by ECM remodeling and increased deposition of FN.

Notably, FN expression differed among asphyxial subtypes. Smothering and strangulation showed the highest levels [23]. By contrast, hanging, drowning, and chemical asphyxia displayed comparatively lower, though still significant, levels of FN expression. This variability may depend on the duration and dynamics of hypoxia, the contribution of concomitant mechanical and vascular factors, and the degree of systemic inflammatory activation at the time of death [24,25].

From a forensic perspective, these observations hold particular relevance. Identifying FN upregulation as a consistent feature of asphyxial deaths may help in the search for objective histological and molecular markers to support the often difficult diagnosis of asphyxia at autopsy. Although traditional morphological signs of asphyxia are well described, they remain nonspecific and may overlap with other causes of sudden death [1,26,27].

Including immunohistochemical markers like FN in forensic workflows could improve diagnostic accuracy, especially when the context is unclear or evidence is limited. The variability in group sample sizes, while partly inevitable in forensic studies, may have influenced the statistical robustness of subgroup analyses.

Furthermore, we consistently found increased FN expression in asphyxial deaths. However, FN is not unique to asphyxia, as similar upregulation also appears in ischemic cardiomyopathy and inflammatory heart disease [28]. Differentiating asphyxial death from sudden cardiac death (SCD) is challenging when the deceased has underlying cardiac disease. A 2022 forensic study observed that in such cases, traditional autopsy and microscopy cannot distinguish asphyxia from SCD because there are no specific morphological features for either. The authors employed Fourier-transform infrared (FTIR) spectroscopy to analyse lung tissue and built classification models capable of distinguishing asphyxia from SCD, even after decomposition. The study highlights the limitations of morphology-based diagnosis and the need for biochemical techniques [29].

The limitations of morphology-based diagnosis have prompted research into biochemical markers of hypoxia. Among the molecules studied, hypoxia-inducible factors, aquaporins (AQPs), cytokines (IL-1β, IL-10, IL-15), surfactant proteins, miRNAs, and metabolic profiles have shown promise. However, the specificity and stability of many markers are still unproven, and postmortem degradation complicates interpretation. FTIR spectroscopy offers a rapid and inexpensive way to distinguish biochemical patterns in lung tissue [29], but it requires specialised equipment and further validation. Researchers explored IHC of neck skin to determine the vitality of ligature marks: tryptase, CD15, IL-15, AQP3, cathepsin D, and FN are effective in detecting early inflammatory or haemostatic responses in neck compression. However, IHC results may be influenced by supra-vital reactions, autolysis, and sampling time [18], necessitating cautious interpretation. A systematic review of IHC analyses of ligature marks found that FN, together with cathepsin D and P-selectin, showed strong positivity in the dermis of vital ligature marks. In experimental studies, Legaz and colleagues observed an extreme positive reaction of FN in the dermis of vital ligature marks. In contrast, cathepsin D showed increased granular staining, and P-selectin showed only weak positivity [18,30]. The same review emphasised that FN's role is to promote wound healing by facilitating cell migration and ECM formation [18].

Another study compared ligature marks with bruises and found reduced FN levels in ligature marks, with this difference particularly

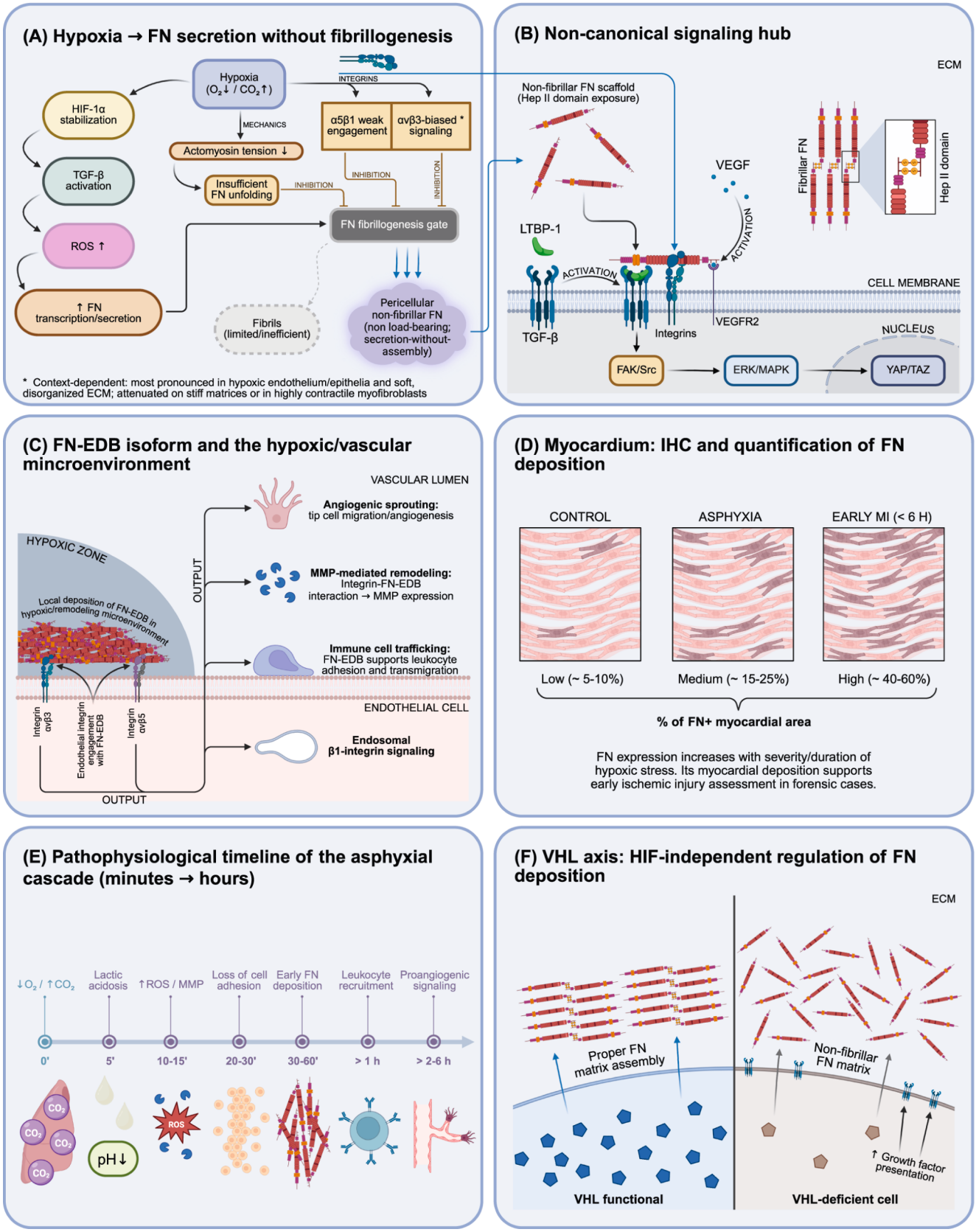

Fig. 2. Fibronectin (FN) plasticity under microenvironmental stress and regulatory imbalance. (A) Hypoxia induces FN secretion via HIF-1α, TGF-β, and ROS, but limits fibrillogenesis. (B) Non-fibrillar FN presents VEGF and TGF-β, boosting FAK → MAPK → YAP signaling; (C) FN-extra domain B isoform (FN-EDB) accumulates in hypoxic vessels, activating αv- and endosomal β1-integrins. (D) FN staining increases from control to asphyxia to early myocardial infarction (MI). (E) Timeline links hypoxia to extracellular matrix (ECM) disruption and inflammation. (F) VHL loss impairs FN assembly independently of hypoxia. Created in BioRender. D'Anna, T. (2025) https://BioRender.com/hkjvj76.

notable in hanging cases [1,31]. The reduction may reflect compression-induced dehydration and reduced plasma extravasation, distinguishing ligature marks from blunt trauma.

Research on mechanical strangulation models has shown that immunohistochemical markers – including tryptase, IL-15, CD15, major histocompatibility complex II (MHC-II), CD1a, AQP1/AQP3, and FN – can detect early inflammatory reactions before overt vascular changes occur [18,32]. These markers may establish the antemortem origin of ligature marks even when death occurs rapidly. FN is advantageous because it is a component of the ECM and may be less susceptible to early postmortem degradation.

Looking forward, forensic pathology is moving toward a molecular era. In addition to FN, several biomarkers are under investigation: AQP3 and AQP5 as indicators of antemortem dehydration in ligature marks, cytokine profiles (IL-1β, IL-10, IL-15) in drowning, surfactant proteins (SP-A and SP-D) and apolipoproteins in drowning, miRNAs such as miRNA-122 and miRNA-3185, and metabolomic signatures. FTIR spectroscopy and mass spectrometry-based metabolomics can differentiate asphyxia from SCD and other causes of death [29]. However, these techniques require further validation, and there is still no single marker that definitively diagnoses asphyxia [33].

Future research should focus on standardised sampling and fixation protocols to minimise variability in immunohistochemical staining and reduce false positives/negatives, multiplex marker panels combining FN with other inflammatory, vascular and metabolic markers to increase sensitivity and specificity, time-course studies examining marker expression at different postmortem intervals under controlled environmental conditions, integration of biochemical data with scene and circumstantial evidence to build probabilistic models that assist forensic experts in court.

5. Strengths and limitations

This study sheds light on FN expression in asphyxial versus traumatic deaths, reinforcing its potential value as a forensic biomarker of hypoxia. However, we acknowledge several limitations that may have influenced the interpretation of our findings.

Because of its retrospective design, the study had limited clinical and circumstantial information, and the small, heterogeneous subgroups of asphyxia reduced the strength of subgroup comparisons. Postmortem interval variability could have influenced protein preservation, despite the application of exclusion criteria. We restricted sampling to the posterior wall of the left ventricular myocardium, which prevented us from assessing regional differences in FN expression. Finally, the absence of a multiparametric panel combining FN with other hypoxia-related markers restricted the ability to evaluate additive or synergistic diagnostic value.

Future studies should focus on larger cohorts, improved image analysis tools, and additional hypoxia-related markers to confirm the specificity and reliability of FN in forensic practice. Our work represents a pilot study, and we are aware of its intrinsic limitations related to sample size; nonetheless, we intend to broaden the casuistry in future investigations. Despite these limitations, we believe that assessing FN might become a valuable addition to the diagnostic workup of asphyxial deaths, helping to better interpret and stratify complex cases.

6. Conclusions

Our findings expand current knowledge on forensic biomarkers of asphyxial deaths by providing controlled comparative data on FN expression in cardiac tissues from asphyxial versus traumatic fatalities. FN immunohistochemistry should be regarded as a complementary tool rather than a standalone diagnostic marker.

In conclusion, our findings highlight FN as a promising marker in asphyxial deaths, showing also distinct expression patterns depending on the underlying hypoxic mechanism. These results not only reinforce the role of FN as a key player in the myocardial response to oxygen deprivation but also open new perspectives for its application as a forensic biomarker.

7. Permissions

Fig. 2 *"Fibronectin (FN) plasticity under microenvironmental stress and regulatory imbalance"* was created by the first author (T.D.), using the BioRender platform, under a BioRender Academic Publication License. The image is licensed under Creative Commons Attribution (CC-BY) for open-access use, in accordance with BioRender's Terms of Service and Academic License Terms. Complete information regarding license terms, image accessibility, and conditions of use is available at the following link: https://BioRender.com/hkjvj76.

8. Data availability statement

The data supporting the findings of this study are not publicly available due to ethical and privacy restrictions, as they involve patient-related clinical and histopathological information. Access to anonymized data may be provided upon reasonable request and with approval from the University Hospital of Palermo Ethical Review Board.

CRediT authorship contribution statement

Tommaso D'Anna: Conceptualization, Investigation, Resources, Writing – original draft. **Rosario Barranco:** Resources, Investigation, Methodology. **Simona Merighi:** Data curation, Formal analysis, Visualization, Software, Writing – original draft. **Valeria Cancila:** Data curation, Validation, Supervision, Writing – original draft. **Ginevra Malta:** Methodology, Resources. **Stefania Zerbo:** Methodology, Resources. **Giuseppe Davide Albano:** Formal analysis, Resources. **Giuseppe Di Pietrantonio:** Visualization. **Isabella Caristo:** Visualization. **Francesco Ventura:** Resources, Supervision, Methodology, Formal analysis. **Antonina Argo:** Conceptualization, Resources, Supervision, Methodology. **Beatrice Belmonte:** Investigation, Resources, Supervision, Data curation, Validation, Project administration.

Funding

This research received no external funding.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] M.A. Sacco, I. Aquila, Post mortem molecular biomarkers of asphyxia: a literature review, Int. J. Mol. Sci. 25 (21) (2024) 11607, https://doi.org/10.3390/ijms252111607.
[2] E.A. Lenselink, Role of fibronectin in normal wound healing, Int. Wound J. 12 (3) (2015) 313–316, https://doi.org/10.1111/iwj.12109.
[3] J.E. Schwarzbauer, D.W. DeSimone, Fibronectins, their fibrillogenesis, and in vivo functions, Cold Spring Harb. Perspect. Biol. 3 (7) (2011) a005041, https://doi.org/10.1101/cshperspect.a005041.
[4] C.J. Dalton, C.A. Lemmon, Fibronectin: molecular structure, fibrillar structure and mechanochemical signaling, Cells 10 (9) (2021) 2443, https://doi.org/10.3390/cells10092443.
[5] M.K. Rana, J. Srivastava, M. Yang, C.S. Chen, D.L. Barber, Hypoxia increases the abundance but not the assembly of extracellular fibronectin during epithelial cell transdifferentiation, J. Cell Sci. 128 (6) (2015) 1083–1089, https://doi.org/10.1242/jcs.155036.
[6] M.M. Sofroniou, C.A. Lemmon, Mechanics and synergistic signaling of fibronectin, integrins, and TGF-β isoforms, Fibrosis 3 (1) (2025) 10003, https://doi.org/10.70322/fibrosis.2025.10003.
[7] G. Guerrero-Barberà, N. Burday, M. Costell, Shaping oncogenic microenvironments: contribution of fibronectin, Front. Cell Dev. Biol. 12 (2024) 1363004, https://doi.org/10.3389/fcell.2024.1363004.

[8] T.C. Lin, C.H. Yang, L.H. Cheng, et al., Fibronectin in cancer: friend or foe, Cells 9 (1) (2019) 27, https://doi.org/10.3390/cells9010027.
[9] F. Zhou, J. Sun, L. Ye, et al., Fibronectin promotes tumor angiogenesis and progression of non-small-cell lung cancer by elevating WISP3 expression via FAK/MAPK/ HIF-1α axis and activating wnt signaling pathway, Exp. Hematol. Oncol. 12 (2023) 61, https://doi.org/10.1186/s40164-023-00419-w.
[10] Y. Zhou, T. Chen, Y. Pan, J. Liu, Exploring the mechanism of fibronectin extra domain B in the tumor microenvironment and implications for targeted immunotherapy and diagnostics (Review), Mol. Med. Rep. 31 (6) (2025) 160, https://doi.org/10.3892/mmr.2025.13525.
[11] R. Valle-Tenney, D.L. Rebolledo, K.E. Lipson, E. Brandan, Role of hypoxia in skeletal muscle fibrosis: synergism between hypoxia and TGF-β signaling upregulates CCN2/CTGF expression specifically in muscle fibers, Matrix Biol. 87 (2020) 48–65, https://doi.org/10.1016/j.matbio.2019.09.003.
[12] H.A. Bluyssen, M.P. Lolkema, M. van Beest, et al., Fibronectin is a hypoxia-independent target of the tumor suppressor VHL, FEBS Lett. 556 (1–3) (2004) 137–142, https://doi.org/10.1016/s0014-5793(03)01392-9.
[13] L. Taglieri, T. Nardo, R. Vicinanza, et al., Thyroid hormone regulates fibronectin expression through the activation of the hypoxia inducible factor 1, Biochem. Biophys. Res. Commun. 493 (3) (2017) 1304–1310, https://doi.org/10.1016/j.bbrc.2017.09.169.
[14] S.A. Ochsner, N.J. McKenna, No dataset left behind: mechanistic insights into thyroid receptor signaling through transcriptomic consensome meta-analysis, Thyroid 30 (4) (2020) 621–639, https://doi.org/10.1089/thy.2019.0307.
[15] Z. Luo, M. Tian, G. Yang, et al., Hypoxia signaling in human health and diseases: implications and prospects for therapeutics, Signal Transduct. Target. Ther. 7 (1) (2022) 218, https://doi.org/10.1038/s41392-022-01080-1.
[16] O.M. Isailă, O.M. Ion, R. Luta, et al., Postmortem immunohistochemical findings in early acute myocardial infarction: a systematic review, Int. J. Mol. Sci. 25 (14) (2024) 7625, https://doi.org/10.3390/ijms25147625.
[17] R. Barranco, C. Castiglioni, F. Ventura, T. Fracasso, Immunohistochemical expression of P-selectin, SP-A, HSP70, aquaporin 5, and fibronectin in saltwater drowning and freshwater drowning, Int. J. Legal Med. 133 (5) (2019) 1461–1467, https://doi.org/10.1007/s00414-019-02105-1.
[18] G. Mansueto, A. Feola, P. Zangani, et al., A clue on the skin: a systematic review on immunohistochemical analyses of the ligature mark, Int. J. Environ. Res. Public Health 19 (4) (2022 Feb 11) 2035, https://doi.org/10.3390/ijerph19042035.
[19] R.O. Hynes, The extracellular matrix: not just pretty fibrils, Science 326 (5957) (2009) 1216–1219, https://doi.org/10.1126/science.1176009.
[20] R. Pankov, K.M. Yamada, Fibronectin at a glance, J. Cell Sci. 115 (Pt 20) (2002) 3861–3863, https://doi.org/10.1242/jcs.00059.
[21] C.I. White, M.A. Jansen, K. McGregor, et al., Cardiomyocyte and vascular smooth muscle-independent 11β-hydroxysteroid dehydrogenase 1 amplifies infarct expansion, hypertrophy, and the development of heart failure after myocardial infarction in male mice, Endocrinology 157 (1) (2016) 346–357, https://doi.org/10.1210/en.2015-1630.
[22] D. Labrousse-Arias, A. Martínez-Ruiz, M.J. Calzada, Hypoxia and redox signaling on extracellular matrix remodeling: from mechanisms to pathological implications, Antioxid. Redox Signal. 27 (12) (2017) 802–822, https://doi.org/10.1089/ars.2017.7275.
[23] S. Yu, T. Mochizuki, T. Katoh, et al., Hypocapnia delays subsequent bupivacaine cardiotoxicity in rats under sevoflurane anesthesia, Springerplus 3 (2014) 371, https://doi.org/10.1186/2193-1801-3-371.
[24] J. Cortis, M.A. Rothschild, Response to Byard's letter "traumatic asphyxia in the young", Int. J. Legal Med. 129 (5) (2015) 1111, https://doi.org/10.1007/s00414-015-1223-1.
[25] P. Saukko, B. Knight, Knight's Forensic Pathology, 4th ed., CRC Press, Boca Raton, 2015.
[26] R.W. Byard, Issues in the classification and pathological diagnosis of asphyxia, Aust. J. Forensic Sci. 43 (1) (2011) 27–38, https://doi.org/10.1080/00450618.2010.482107.
[27] M. Scopetti, M. Padovano, F. Manetti, et al., Molecular autopsy in asphyxia deaths: diagnostic perspectives of miRNAs in the evaluation of hypoxia response, Int. J. Med. Sci. 20 (6) (2023) 749–753, https://doi.org/10.7150/ijms.79539.
[28] N.G. Frangogiannis, The extracellular matrix in ischemic and nonischemic heart failure, Circ. Res. 125 (1) (2019) 117–146, https://doi.org/10.1161/CIRCRESAHA.119.311148.
[29] K. Zhang, R. Liu, Y. Tuo, et al., Distinguishing asphyxia from sudden cardiac death as the cause of death from the lung tissues of rats and humans using Fourier transform infrared spectroscopy, ACS Omega 7 (50) (2022) 46859–46869, https://doi.org/10.1021/acsomega.2c05968.
[30] I. Legaz, M.P. Pérez-Cárceles, M. Gimenez, et al., Immunohistochemistry as a tool to characterize human skin wounds of hanging marks, Rom J Leg Med 26 (4) (2018) 354–358, https://doi.org/10.4323/rjlm.2018.354.
[31] I. Legaz Pérez, M. Falcón, M. Gimenez, et al., Diagnosis of vitality in skin wounds in the ligature marks resulting from suicide hanging, Am. J. Forensic Med. Pathol. 38 (3) (2017) 211–218, https://doi.org/10.1097/PAF.0000000000000322.
[32] A.C. Ros, S. Bacci, A. Luna, I. Legaz, Forensic impact of the omics science involved in the wound: a systematic review, Front Med (lausanne) 8 (2022) 786798, https://doi.org/10.3389/fmed.2021.786798.
[33] A. Porzionato, R. Boscolo-Berto, Assessing violent mechanical asphyxia in forensic pathology: state-of-the-art and unanswered questions, Adv. Clin. Exp. Med. 33 (9) (2024) 905–908, https://doi.org/10.17219/acem/192226.