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Cytological-Pathologic Correlation

Pulmonary intravascular mononuclear cell accumulation in cases of mechanical asphyxia due to external neck compression

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ABSTRACT

Pulmonary intravascular mononuclear cell accumulations have been described in mechanical asphyxia, but their diagnostic value and independence from demographic or post-mortem factors remain uncertain. This study assessed the frequency of these accumulations in asphyxial deaths due to external neck compression compared with non-asphyxial deaths, and evaluated whether the association persists after adjustment for potential confounders.

Lung tissue samples from 31 external neck-compression deaths and 151 non-asphyxial controls were examined histologically. A subset underwent immunohistochemical phenotyping. Univariable comparisons were performed using χ^2 and Mann–Whitney *U* tests. A multivariable logistic regression model—including age, sex, post-mortem interval (PMI), and body mass index (BMI)—was used to evaluate the independence of the association.

Intravascular mononuclear accumulations were observed in 41.9 % of neck-compression deaths versus 17.2 % of controls ($p < 0.01$; unadjusted OR 3.47, 95 % CI 1.52–7.96). In the multivariable model, external neck compression remained independently associated with the presence of intravascular accumulations (adjusted OR 2.93, 95 % CI 1.14–7.52; $p = 0.025$), while age, sex, PMI, and BMI showed no significant effect. Immunohistochemistry confirmed that accumulations consisted of mature mononuclear cell subsets.

Pulmonary intravascular mononuclear accumulations occur significantly more often in asphyxial deaths involving external neck compression, and this association persists after adjustment for key demographic and post-mortem variables. Although not specific to mechanical asphyxia, these accumulations represent a practical ancillary marker that may support the diagnosis of neck-compression vitality, especially in cases with limited external findings.

1. Introduction

The post-mortem diagnosis of mechanical asphyxia due to neck compression remains one of the most debated issues in forensic pathology, especially when external signs are subtle or absent. In such cases, histological and immunohistochemical approaches can provide useful ancillary evidence.

Over the years, several pulmonary alterations have been described, including emphysema and oedema [1,2], intra-alveolar and interstitial haemorrhages [3], macrophage activation and giant cell formation [4], mast cell distribution [5,6], and the expression of proteins linked to hypoxia such as surfactant protein A (SP-A) and hypoxia-inducible factor 1-α (HIF-1α) [7,8].

Among less explored features, intravascular accumulations of leukocytes and immature cells were first reported in strangulation and hanging by Brinkmann [9] and Grellner & Madea [10]. These were proposed as potential vitality indicators, but their specificity and reproducibility have been debated. Recent studies have emphasized the importance of adopting a multiparametric approach, integrating histological, immunohistochemical, biochemical, and molecular markers to strengthen the evaluation of vital reactions in asphyxia [8,11-14].

Within this framework, pulmonary intravascular mononuclear cell

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accumulations deserve renewed attention. Experimental models have shown that catecholamine surges and acute hypoxia can mobilize leukocytes and promote their redistribution to the lung vasculature [15-18]. These mechanisms suggest that such findings may represent a vital reaction, reinforcing their potential forensic value. The present study aims to evaluate the frequency and cellular composition of intravascular mononuclear accumulation in asphyxial deaths due to external neck compression compared with controls, and to assess their role as ancillary markers of vitality.

2. Materials and methods

2.1. Cases and sample collection

The study included 31 cases of asphyxial death due to external neck compression examined at the Institute of Legal Medicine, University of Modena and Reggio Emilia between 1994 and 2024. The control group consisted of 151 non-asphyxial deaths, including cardiac arrhythmia, cerebral haemorrhage, non-pulmonary cancer, myocardial infarction, acute heart failure, and intoxications. Cases with evidence of pulmonary pathology or resuscitation maneuvers were excluded. Sample characteristics are summarized in Table 1.

During autopsy, lungs were inspected and weighed. No traumatic or chronic pulmonary disease was observed. Tissue samples were collected from peripheral areas of the upper lobes, fixed in buffered 4 % formaldehyde, dehydrated, and paraffin-embedded.

2.2. Histology

Sections of 5 μm were stained with Hematoxylin and Eosin (H&E). A blinded forensic pathologist with >10 years' experience screened the slides at 10× magnification, with confirmation at higher magnification (20–40×). Intravascular mononuclear cell accumulations were recorded when either compact aggregates or dispersed mononuclear cells were observed within vessel lumina, regardless of their extension.

Table 1
Sample characteristics. PMI = Post-Mortem Interval; BMI = Body Mass Index.

Cause of death	Nr. of cases	Sex (M/F)	Mean age (years)	Age range (years)	Mean PMI (days)	Mean BMI (kg/m^2)
External neck compression asphyxia (manual of ligature strangulation)	31	14/17	39.12	1–90	3.55	23.81
Control group (non-asphyxial deaths)						
Primary cardiac arrhythmia	29	20/9	54.45	21–93	1.67	24.93
Gunshot wounds to the head, subarachnoid haemorrhage, or cerebral/cerebellar haemorrhage	28	24/4	56.55	0–92	2.87	22.63
Non-pulmonary cancer	27	23/4	52.86	0–93	1.83	24.80
Acute heart failure	24	17/7	63.92	30–91	1.52	25.84
Myocardial infarction	22	16/6	60.64	43–81	1.72	25.72
Acute intoxication (drugs, medicines, or alcohol)	21	16/5	49.73	11–88	2.26	23.71
Total control group	151	117/34	56.31	0–93	1.99	24.57

2.3. Immunohistochemistry

A subset of 10 asphyxial and 10 non-asphyxial cases showing intravascular mononuclear cells underwent immunohistochemistry (see Tables 2 and 3). Sections (4 μm) were processed with heat-induced epitope retrieval, peroxidase blocking, and overnight incubation with primary antibodies (see Table 2 for clones, producers, and dilutions). Detection was performed with the EXPOSE Mouse and Rabbit Specific HRP/DAB kit (Abcam). Slides were counterstained with Hematoxylin and mounted. Negative controls were run by omitting primary antibodies.

Immuno-stained slides were evaluated in blinded fashion. Fields were screened at 10× and examined at higher magnification (20–40×). Immuno-positive cells were counted manually in duplicate, and results expressed as percentage of positive cells over the total cell count in each field. Data are presented as mean ± SD.

2.4. Statistical analysis

Group comparisons were performed using χ^2 test for categorical variables and Mann–Whitney *U* test for continuous variables, with significance set at $p < 0.05$. Odds ratios (OR) with 95 % confidence intervals (Cis) were calculated to quantify the association between intravascular accumulations and cause of death.

Categorical data (presence vs absence of intravascular mononuclear cell accumulations) were compared between neck-compression asphyxial deaths and non-asphyxial controls using the χ^2 test, and ORs with 95 % CIs were calculated. In addition, a multivariable logistic regression model was constructed with the presence of intravascular accumulations as the dependent variable and cause-of-death category (neck compression vs controls), age, sex, post-mortem interval (PMI), and body mass index (BMI) as covariates. Results are reported as adjusted ORs with 95 % CIs. A secondary model restricted to non-asphyxial deaths was fitted to explore potential associations between demographic/post-mortem variables and the marker within the control group.

3. Results

3.1. Histology

Intravascular mononuclear cell accumulations appeared either as compact aggregates partially or completely occluding the vascular lumen, or as dispersed mononuclear elements intermingled with erythrocytes (Fig. 1).

3.2. Univariable analysis

Intravascular mononuclear cell accumulations were present in 13/31 (41.9 %) neck-compression deaths and in 26/151 (17.2 %) non-asphyxial controls. This yielded an unadjusted odds ratio of 3.47 (95

Table 2
Panel of primary antibodies used for immunohistochemical phenotyping.

Primary antibody	Target cell type	Dilution
Anti-CD5 (mRb, SP19, Thermo Scientific)	T-lymphocytes and thymic precursors	1:50
Anti-CD8 (mRb, SP16, Thermo Scientific)	Cytotoxic T-lymphocytes	1:50
Anti-CD16 (mMs, DJ130c, Santa Cruz Biotechnology)	NK cells and macrophages	1:250
Anti-CD20 (pRb, Thermo Scientific)	B-lymphocytes	1:400
Anti-CD34 (mMs, QBEnd/10, Thermo Scientific)	Immature hematopoietic progenitors	1:400
Anti-CD64 (mMs, 3D3, Abcam)	Monocytes and macrophages	1:150

Abbreviations: mRb, monoclonal rabbit; mMs, monoclonal mouse; pRb, polyclonal rabbit.

Table 3
Immunohistochemical protocol (Abcam EXPOSE HRP/DAB detection kit).

Step	Reagent	Duration/Conditions
Antigen retrieval	Citrate buffer pH 6.0	3 × 5 min (microwave oven)
Peroxidase blocking	Hydrogen Peroxide Block (kit)	10 min, RT, humid chamber
Blocking nonspecific sites	Protein Block (kit)	30 min, RT, humid chamber
Primary antibody incubation	Anti-human (rabbit/mouse)	o/n at 4 °C + 30 min at RT, humid chamber
Mouse-specific reagent	Specifying reagent (kit)	10 min, RT, humid chamber
Secondary antibody	Goat anti-rabbit conjugate	15 min, RT, humid chamber
Chromogen development	DAB (3,3′-diaminobenzidine)	5 min, RT, humid chamber
Nuclear counterstain	Hematoxylin	2 min, RT
Rinse	Running tap water	5 min, RT

Abbreviations: RT, room temperature; o/n, overnight.

% CI 1.52–7.96; $p = 0.003$), indicating that such accumulations were more than three times as likely in deaths due to external neck compression.

3.3. Multivariable analysis

In the multivariable logistic regression model including age, sex, PMI, and BMI, neck-compression asphyxia remained independently associated with the presence of intravascular mononuclear cell accumulations (adjusted OR 2.93; 95 % CI 1.14–7.52; $p = 0.025$). None of the covariates (age, sex, PMI, BMI) showed a statistically significant association with the marker (all $p > 0.30$).

When the analysis was restricted to non-asphyxial deaths, age, sex, PMI, and BMI again showed no significant association with the presence of intravascular mononuclear accumulations (all $p > 0.17$), suggesting that these variables do not strongly influence the occurrence of the marker in the control population.

3.4. Immunohistochemistry

In the subset analysis (10 asphyxial, 10 control cases), immunohistochemistry confirmed the presence of multiple mononuclear subtypes (Fig. 2): T-lymphocytes (CD5+, CD8+), B-lymphocytes (CD20+), NK cells/macrophages (CD16+), and monocytes/macrophages (CD64+). No CD34+ immature myeloid cells were detected in either group.

Quantitative analysis showed comparable distributions of cell subtypes between groups. Mean values in the asphyxial vs. control group

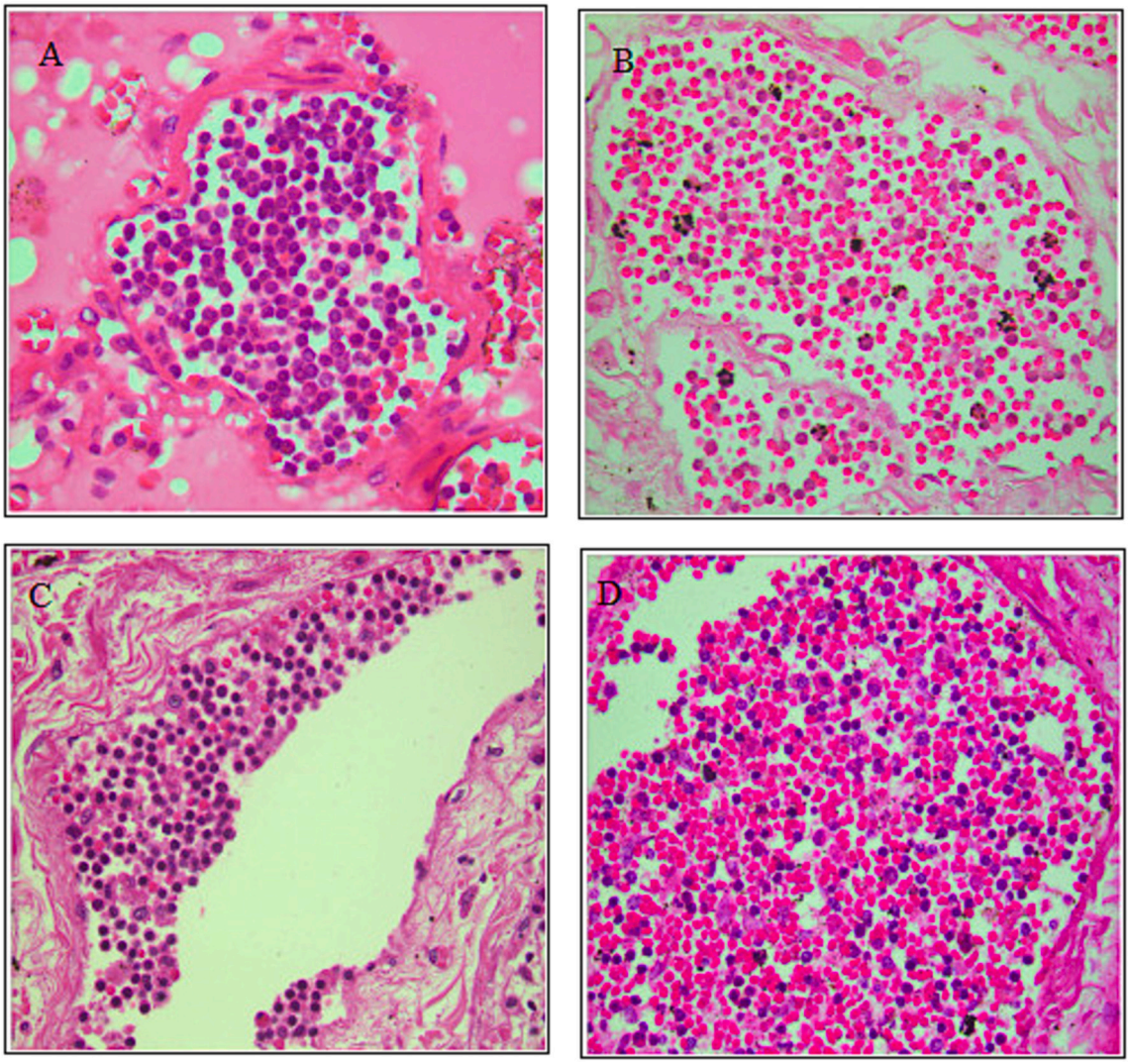

Fig. 1. Alterations of lung vascular content observed in the asphyxia group (b) and in the non-asphyxia group (c, d). Mononuclear cells, with no poly-lobulated nuclei, detected in pulmonary vessels arranged to aggregates (a, c) or scattered diffusely between several erythrocytes (b, d). (HE, original magnification 40×).

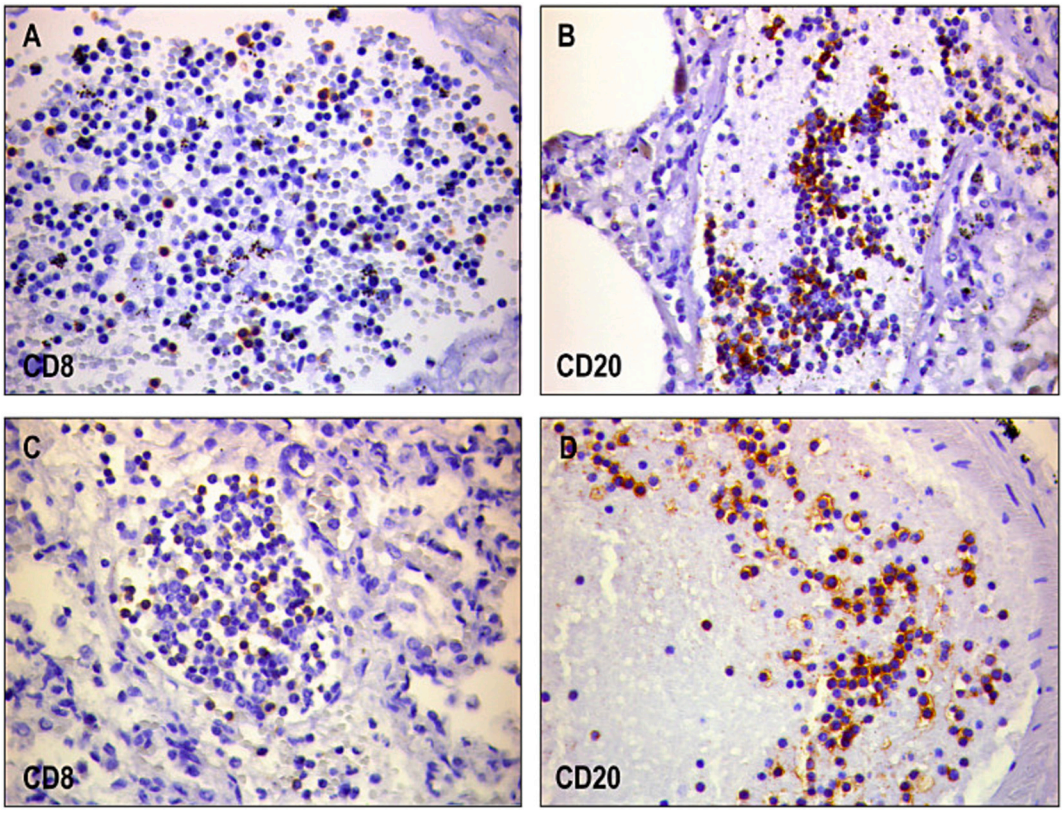

Fig. 2. A representative pattern of CD8 and CD20 immunostaining in lung vessels in non-asphyxia cases (a, b) and in asphyxia cases (40×) (c, d).

were: CD5 (T-lymphocytes) 11.1 % vs. 14.8 %, CD8 (cytotoxic T-lymphocytes) 6.4 % vs. 6.9 %, CD16 (NK cells/macrophages) 7.8 % vs. 17.7 %, CD20 (B-lymphocytes) 9.5 % vs. 14.1 %, CD64 (monocytes/macrophages) 5.5 % vs. 11.1 % (all $p > 0.05$; see Table 4).

4. Discussion

This study contributes to the growing body of evidence on pulmonary vital reactions and, to our knowledge, represents one of the largest case–control series on intravascular mononuclear cell accumulations in forensic settings. Our result show that pulmonary intravascular mononuclear cell accumulations occur significantly more often in deaths due to external neck compression (41.9 %) than in non-asphyxial controls (17.2 %). These findings extend earlier observations [9,10] and confirm that the phenomenon reflects a vital reaction. Unlike previous reports, no CD34-positive precursors were observed, indicating that in our cohort the process predominantly involves mature immune cells.

The absence of significant differences in the immunophenotypic profile suggests that the forensic utility of this feature depends on its occurrence as a whole, rather than on the representation of individual immune subpopulations.

The biological plausibility of our results is supported by experimental evidence showing that acute catecholamine surges induce rapid leukocyte mobilization [15,17] and that hypoxia enhances pulmonary leukocyte sequestration [16,18]. Moreover, leukocyte margination and endothelial damage represent additional mechanisms that may contribute to this phenomenon: hypoxia and mechanical vascular stress can trigger endothelial activation, increasing adhesion molecule expression (ICAM-1, P-selectin) and thereby favoring intravascular accumulation [7].

In the present study, the association between external neck compression and pulmonary intravascular mononuclear accumulations persisted even after adjustment for major demographic and post-mortem variables. In the multivariable logistic regression model including age, sex, PMI, and BMI, external neck compression remained an independent predictor of intravascular accumulations, with an adjusted odds ratio similar in magnitude to the unadjusted estimate. None of the covariates showed a significant association with the marker, and analyses restricted to non-asphyxial deaths likewise revealed no relationship between demographic or post-mortem variables and the presence of intravascular mononuclear cells.

Although residual confounding from unmeasured variables cannot be excluded, the consistency between univariate and multivariable models strengthens the interpretation of intravascular mononuclear accumulations as a vitality-related phenomenon more frequently encountered in deaths involving external neck compression. From a forensic perspective, the relevance of these findings lies in their accessibility. Unlike molecular biomarkers that require advanced technologies [11], pulmonary intravascular accumulations can be assessed on routine H&E sections. This makes them particularly valuable in everyday practice and in resource-limited settings. Other ancillary markers, such as SP-A [1,2], HIF-1α [7,8], mast cell activation [5,6], and inflammatory signatures [3], further support a multiparametric approach where complementary data are integrated for greater diagnostic certainty. Imaging-based techniques, such as post-mortem CT densitometry, may further complement histological evaluation [19].

Table 4
Percentage of immune-positive intravascular cells in the lungs of asphyxial and non-asphyxial deaths (mean ± SD).

Antibody	Asphyxial deaths (mean ± SD, %)	Non-asphyxial deaths (mean ± SD, %)
CD5	11.1 ± 3.2	14.8 ± 21.8
CD8	6.4 ± 5.6	6.9 ± 4.5
CD16	7.8 ± 6.5	17.7 ± 16.7
CD20	9.5 ± 6.3	14.1 ± 15.9
CD64	5.5 ± 4.5	11.1 ± 9.4

The main limitations of this study include the relatively small immunohistochemical subgroup, lack of systematic control for post-mortem interval and agonal duration, and possible variability in antigen retrieval. Larger multicenter series with standardized protocols and digital image analysis are needed to validate and refine these observations.

5. Conclusions

Pulmonary intravascular mononuclear cell accumulations are significantly more frequent in deaths due to external neck compression than in controls. Their occurrence likely reflects combined mechanisms of leukocyte redistribution, hypoxia-driven margination, and endothelial activation. While not specific to mechanical asphyxia, they represent a reliable and reproducible marker of vitality that can be recognized on routine histology.

These findings support the integration of intravascular mononuclear accumulations into everyday forensic protocols, particularly in cases where external signs are minimal or absent, and reinforce the importance of adopting a multiparametric approach to the post-mortem diagnosis of mechanical asphyxia.

CRediT authorship contribution statement

Anna Laura Santunione: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Jessika Camatti:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Silvia Corradi:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Enrico Silingardi:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Rossana Cecchi:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Patient consent

Not applicable. All subjects included in the study were deceased. All data were fully anonymised, and no identifying information is reported.

Ethical approval

This study was a retrospective forensic autopsy study conducted on deceased individuals only. According to national legislation and institutional policies, ethical committee approval is not applicable for retrospective studies based exclusively on anonymized autopsy material.

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Declaration of competing interest

The authors declare no competing interests.

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Data availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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