G91 A Methodological Approach in Deep Venous Thrombosis Fatal Cases: Clinical Diagnosis, Therapy, Genetics, and a Histopathological Approach

Irene Riezzo, MD, PhD, University of Foggia, Department of Forensic Pathology, Ospedale "C. D'Avanzo", Viale degli Aviatori, 1, Foggia, 71100, ITALY; Dania De Carlo, MD, and Santina Cantatore, MLT, University of Foggia, Department of Forensic Pathology Viale degli Aviatori 1, Foggia, 71100, ITALY; Francesco Ventura, MD, University of Genova, Department of Forensic Pathology, via de' Toni, 12, Genova, 16132, ITALY; Alessandro Bonsignore, MD, University of Genova, Via de Toni 12, Dept of Legal & Forensic Med, Genova, 16132, ITALY; and Margherita Neri, MD, PhD, University of Foggia, Department Forensic Pathology, Viale degli Aviatori 1, Foggia, 71100, ITALY*

After attending this presentation, attendees will see how clinical diagnosis of pulmonary embolism (PE) is notoriously inaccurate, with many cases either wrongly diagnosed (overdiagnosed) or missed (underdiagnosed), and autopsy is still considered as the diagnostic gold standard. The accuracy of antemortem diagnosis of pulmonary embolism is within the range of just 10–30%, so representing one of the most frequent missed diagnoses in sudden, unexpected death. Abnormalities within the gene loci encoding for natural anticoagulants (antithrombin, protein C, and protein S) and for fibrinogen have been shown to be rather uncommon risk factors for VTE. The goal of this study was to verify the systematic search for the most common genetic thrombophilias (Factor V Leiden (G1691A)) and FII ((G20210A) gene variants) and dating the thrombus.

This presentation will impact the forensic science community by showing how in a case of death from PE, autopsy dissection, documentation and studies concerning pulmonary emboli are relevant for the evaluation of such fatality. The criteria to determine the chronological changes in the venous sites of thrombosis are supported by many technologies and methodologies available to forensic scientists, but are unfortunately very rarely applied in daily judicial practice autopsy findings, histology and genetic studies are essential to analyze various thrombotic risk factors and etiologies; thus a reliable protocol is needed for their investigation. The review of polymorphisms associated with thrombotic disease has highlighted the considerable variability of clinical associations with the various polymorphisms. Histological age determination of thromboses is a valid aid to date the DVT phenomenon and the chronological changes of the thrombus, so the forensic pathologist can objectively determine the causal relationship between a previous trauma and the following fatal PTE episode.

Fourty-three fatal cases of pulmonary embolism as confirmed by postmortem examination are described. The selection was carried out on the basis of some criteria including completeness of patients' medical history diagnosis of certainty of the cause of death, identification of macroscopic thrombus, identification of the thrombotic site, availability of formalin-fixed paraffin-embedded tissues for genetic and histochronologic studies. In all these cases postmortem examination confirmed the diagnosis of PE. PE was recorded as the cause of death only when the necropsy stated that embolism was the main contributing cause of death and when emboli were identified either in the main pulmonary trunk or in the proximal right or left pulmonary arteries formed from the bifurcation of the main trunk. Emboli found in the distal pulmonary arteries after further division of the right and left pulmonary arteries were included too. In each case, five tissue samples were obtained from lungs; one piece from each lobe. Cross sections in each segmental pulmonary artery were prepared and microscopically examined. The dissection of the deep veins of the pelvis and legs was performed to search for the starting point of venous embolism. In the venous sites of thrombosis, the histological assessment was performed in conjunction with the surrounding vascular wall of uncut blood vessel with at least three to six different transverse incisions. Pathologic features were estimated using histological sections stained by hematoxylin–eosin, (H&E), trichromic stains (Masson, Azan, Mallory, PTAH, Van Gieson) and Von Kossa for calcium salts. Perl's stain for hemosiderin was used to confirm the presence of iron. Immunohistochemical investigation of thrombus and embolus samples was performed using polyclonal anti-fibrinogen antibodies, CD61, CD45, CD15, CD68. To investigate whether the FV Leiden and FII mutations increase the risk of fatal PE, we investigated their presence in pathology material. The spectrophotometric analysis of the quality and quantity of DNA which was extracted led us to choose the heart as the best specimen to be used as a reference sample. DNA was extracted from a total of 43 paraffin wax embedded tissue specimens of hearth. For DNA extraction "*Tissue and Hair Extraction Kit (for use with DNA IQ)*" protocol was used. The spectrophotometric analysis of DNA was performed. DNA samples were genotyped by real-time quantitative polymerase chain reaction for factor V Leiden and FII A20210 alleles.

As a whole, 41 patients (95.3%) had at least one risk factor. Pre-existing symptoms are described just before fatal embolism in 18 (41.9%) out 43 patients. In 18 out of 43 (41.9%) it was not possible to find the thrombotic site. In 24 out of the remaining 25 cases the involvement of the deep veins of one leg was shown; in one case the thrombus was localised in the inferior caval vein, 10 (41.7%) were iliac vein thromboses, seven (29.1%) femoral, two (8,3%) popliteal, three (12.6%) posterior-tibial, one (4.1%) anterior-tibial and one (4.1%) peroneal vein thromboses. In our cohort of patients, four (10%) out of 40 cases carried the 20210A prothrombin gene variant in heterozygosis. One (2.5%) out of 40 carried the Factor V Leiden (G1691A) gene variant in heterozygosis. Patients carrying these gene variants in homozygosis or carrying both were not present in our case-series.

This study strongly underlines the relevance of a complete methodological approach, integrating clinical data by means of autopsy findings and histological study. On the contrary, investigating common inherited thrombophilia is not warranted.

 ** Presenting Author*

This observation strengthens the concept that thrombophilia screening is indicated when VTE occurs in young subjects in absence of malignancy, major trauma or surgery.

Factor V Leiden, Fatal Venous Thromboembolism, Prothrombin

 ** Presenting Author*