

## **Forensic medical investigation of military aviators killed in aircraft accidents – new methods and unexpected limitations.**

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**Background:** Casualties from aviation mishaps require particular attention in medico-legal death investigation. All acute and chronic, somatic as well as mental conditions possibly involved in a pilot's sudden incapacitation to fly must be detected. Accidents involving military high-performance jet aircraft frequently cause severe destruction of the victim's body. Hereby, the results from forensic autopsy can be severely limited. New methods, also effective in tissues previously exposed to mechanical disruption, heat, blast, or fire might increase the diagnostic outcome.

**Material and methods:** To provide insight in the pre-mortal tissue hemostasis and physiology of death victims from aircraft accidents, a panel of more than 100 micro-RNAs (miRNAs) and messenger-RNAs (mRNAs) was defined. These molecular markers for cardiocirculatory burden, hypoxia, and cellular stress reactions were implemented using archived tissue specimens from previously healthy pilots killed in mid-air collisions between military jet aircraft. The first study part focused on heart muscle samples. RT-PCR-based assessment was carried out, followed by semiquantitative data analysis using the  $2^{-\Delta\Delta C_t}$  method. Corresponding samples from sudden death cases unrelated to aviation, including road and railway accidents, were used as normal controls. For comprehensive data interpretation, autopsy samples from hospitalized patients, which died of hypertrophic or ischemic heart disease, were co-analyzed.

**Results:** Optimized extraction protocols allowed the detection of RNAs in concentrations down to 0.0002 femtomol per microliter tissue lysate. Up-regulations of miRNAs indicating myocardial hypertrophy between 2.6-fold and 7.5-fold were detected in the jet pilot group compared to normal controls. However, corresponding expression levels up to 5.7-fold were demonstrated in the myocardium of patients which died from hypertrophic heart disease. Comparable overexpression levels of miRNAs indicating myocardial hypoxia were found between jet aviators and casualties from ischemic heart disease. Significant myocardial stress reactions in jet pilots were demonstrated showing up to 33-fold overexpression rates of mRNAs coding for Heat-shock-Protein (HSP)-A4 and HSP-B1 compared to normal controls.

**Discussion:** Molecular genetic analysis successfully supports medico-legal death investigation. However, data from deceased military jet pilots must be carefully interpreted. Markers for myocardial hypertrophy and hypoxic reaction potentially reflect physiologic adaptation to the special burdens of high-performance jet flying rather than true pathologic changes.