



Praca kazuistyczna  
Case reports

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## Immunohistochemical detection of insulin at the injection site in a case of suspected murder with the suicide of the perpetrator

### Immunohistochemiczna detekcja insuliny w miejscu wkłucia w przypadku podejrzenia zabójstwa z samobójstwem sprawcy

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#### Abstract

In medicolegal practice, rare cases involving suicidal, criminal, or accidental insulin overdose are both analytically and forensically challenging. The aim of this study is to present a model procedure in such cases, developed at the Department of Forensic Medicine and Toxicology of the Medical University of Silesia in Katowice, with particular emphasis on the possibility of additional confirmation of insulin intake by its immunohistochemical detection at the injection site. In the example case presented here, an immunohistochemical examination using FLEX Polyclonal Guinea Pig Anti-Insulin antibody (code IR002, Dako) confirmed the presence of insulin in the subcutaneous tissue of the victims. In our opinion, the method of immunohistochemical detection of insulin at the injection site can and should be used routinely in such cases.

**Key words:** insulin overdose, murder-suicide, forensic histopathology, immunohistochemistry

## Streszczenie

W praktyce medyczno-sądowej przypadki związane z samobójczym, zbrodniczym lub przypadkowym przedawkowaniem insuliny są rzadkie i należą do trudnych pod względem analitycznym i opiniodawczym. Celem pracy jest przedstawienie modelowego postępowania w tego typu przypadkach opracowanego w Katedrze i Zakładzie Medycyny Sądowej i Toksykologii Sądowo-Lekarskiej w Katowicach, ze szczególnym uwzględnieniem możliwości dodatkowego potwierdzenia podania insuliny przez jej immunohistochemiczną detekcję w miejscu wkłucia. W zaprezentowanym przypadku badanie immunohistochemiczne z wykorzystaniem przeciwciała Polyclonal Guinea Pig Anti-Insulin FLEX IR002 (Dako) potwierdziło obecność insuliny w tkance podskórnej obu ofiar. Naszym zdaniem metoda immunohistochemicznej detekcji insuliny w miejscu wkłucia może i powinna być stosowana w każdym tego rodzaju przypadku.

**Słowa kluczowe:** przedawkowanie insuliny, zabójstwo-samobójstwo, histopatologia sądowo-lekarska, immunohistochemia

## 1. Introduction

In medicolegal practice, rare cases involving suicidal, criminal, or accidental insulin overdose are both analytically and forensically challenging [1]. In each of these cases, it is crucial to obtain preliminary information from the prosecutor or the police prior to forensic autopsy in order to allow for proper macroscopic examination and targeted post-mortem diagnosis [2].

The aim of this study is to present a model procedure in such cases, developed at the Department of Forensic Medicine and Toxicology of the Medical University of Silesia in Katowice, with particular emphasis on the possibility of additional confirmation of insulin intake by its immunohistochemical detection at the injection site.

## 2. Case report

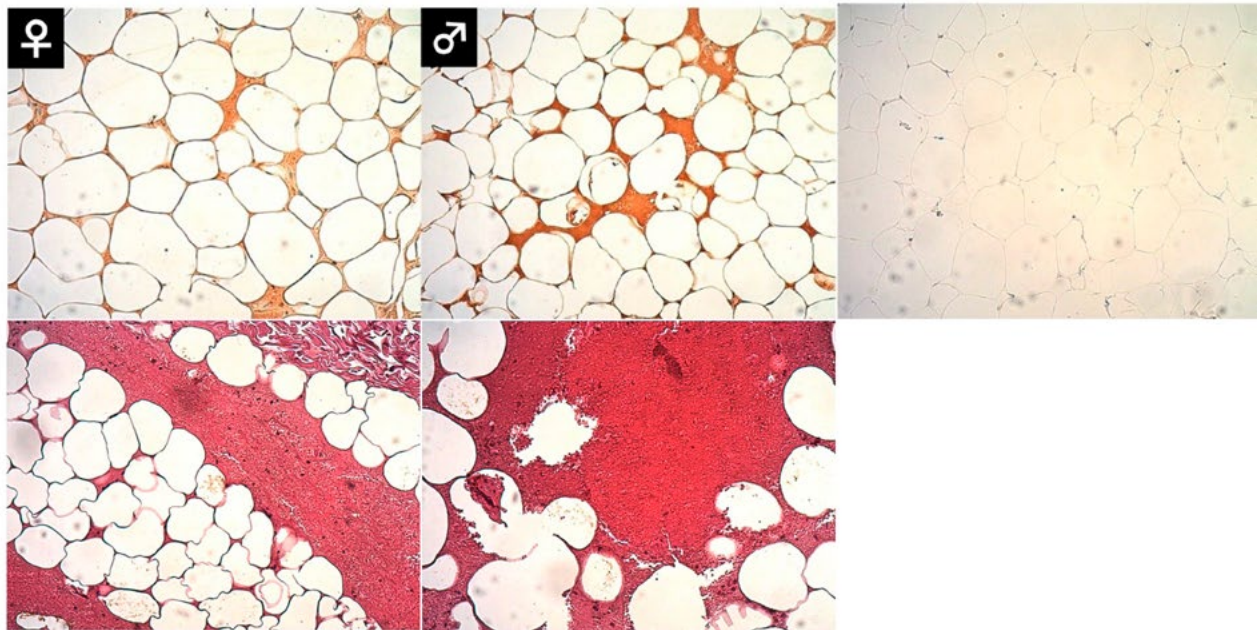
According to the prosecutor's office, a 63-year-old man allegedly killed his wife and dog and then committed suicide. A suicide note, a Penfill insulin injection cartridge, and an empty package of Novo-Mix 30 (a mixture of rapid- and long-acting insulin analogues) were found in the flat. Forensic veterinary autopsy of the dog revealed fatal injuries in the form of deep lacerations of the neck and abdomen, while visual examination and forensic autopsies of

the man and woman failed to reveal the cause of their deaths. However, injection sites were found on the thighs and one arm (Fig. 1). Extensive material was secured for additional biochemical, chemico-toxicological, and histopathological analyses, including sections from injection sites. An immunohistochemical examination was performed using FLEX Polyclonal Guinea Pig Anti-Insulin antibody (code IR002, Dako) according to the manufacturer's instructions. This antibody was developed against porcine pancreatic insulin and had cross-reactivity with human insulin (100% by radioimmunoassay). There is no information about the specificity of this antibody used in immunohistochemical stainings, so the authors performed their local (in-house) validation. The positive control was, of course, B cells of the islets of Langerhans. According to the manufacturer information, this product has been optimized for use on human tissues.

Immunohistochemical examination confirmed the presence of insulin in the subcutaneous tissue of both victims (Fig. 2). The microphotographs present different levels of positive reaction, which is more intense in the man. Taking into account the other data, it can be assumed that the cause of death of both victims was criminal insulin overdose (the husband killed his wife and then committed suicide).



**Figure 1.** Ecchymotic puncture wounds corresponding to injection marks – suicidal insulin injection into the thigh.



**Figure 2.** Positive immunohistochemical reaction for insulin in the subcutaneous tissue between adipocytes at the injection sites in both victims. On the right side, a negative reaction from a distant site (negative control). Relevant micrographs of puncture sites with visible hemorrhages are shown below immunohistochemical images.

### 3. Discussion

The above description concerns a typical case of murder-suicide use of insulin, in which the typical range of post-mortem examinations was extended to immunohistochemical detection of insulin at injection sites. To our knowledge, this was the first time this method had been used in forensic histopathological practice in Poland. There are only a few descriptions of such applications of immunohistochemical examination in the literature, the first of which dates from 1986 [3, 4]. In our opinion, the method of immunohistochemical detection of insulin at the injection site can and should continue to be routinely used in any such case, especially given the current wide availability of immunohistochemical tests in medical practice. The commissioning party (usually the prosecutor) should be informed of such a possibility by the forensic medical examiner or pathologist who conducts the post-mortem examination.

The scheme of diagnostic procedures in cases of criminal use of insulin recommended in Poland was published in 2019 [5]. In post-mortem diagnostics in this type of cases, certain stages are distinguished:

- the pre-autopsy stage (reading the findings of the investigation);
- the stage of an appropriate forensic autopsy with the collection of biological material for additional examinations;
- the stage of additional histopathological examinations, including immunohistochemistry;
- the stage of additional toxicological tests (exclusion of poisoning with xenobiotics);
- the stage of additional genetic tests (analysis of the tool with which the victim was administered insulin);
- the stage of additional biochemical tests (in practice, the concentrations of insulin, C-peptide, glucose, lactic acid, and glycated hemoglobin (HbA1c) are most often used);
- final stage (writing conclusions).

### References

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