Introduction

In contrast to clinical pathology, forensic pathology investigates small scale studies that are often unique to a specific case and are bound by legal restrictions. A forensic pathologist then has to deal with several areas of expertise, also at autopsies. There however is a need for additional tools in forensic autopsies. In this thesis we aimed to improve diagnostics related to I) skin wound age determination, II) taphonomy and III) cardiovascular pathology.

I: Skin wound age determination

For correct diagnosis of an injury, amnestic information of the estimated time of injury is combined with macroscopic and microscopic analysis of the wound. The collection and processing of samples usually involves photographing the wounds and taking either the entire wound or representative samples for microscopic analysis and immunohistochemtical studies (1, 2). Immunohistochemical studies have been reported to be useful in detecting and differentiating between the different inflammatory cells and/or inflammatory markers in wound analysis (3, 4, 5). After the induction of wound injury, an immune response is induced in the process of wound healing, beginning immediately and lasting up to several weeks. This has been reviewed in *Chapter 2*. The aim of this chapter was to introduce the reader to the fact that although much work has been done on injury dating the gold standard as such is yet to be found.

In forensic pathology a crucial question always remains in the dating of wound injuries: Did a wound occur before death or was it a post mortem injury. Therefore determining the vitality of skin wounds is of vital importance. What determines a vital wound? Several histological characteristics such as hemorrhage have been suggested; although these have also been shown to occur in non-vital wounds (6, 7, 8, 10). We wondered whether analysis of markers related to the process of coagulation, which theoretically is induced in hemorrhage of vital wounds, could discriminate between vital and non-vital wounds. This we have studied in *Chapter 3,* including the development of a probability scoring system for wound injury dating.

It is known that the immune response differs between different types of wound injury. This is especially true for burn injuries, as they induce a severe immunological response and if not treated can lead to systematic infection causing death. The acute phase proteins complement and C-reactive protein are known to be increased in plasma up to months after a burn injury (10, 11, 12, 13). Although it is known that these acute phase proteins play a role in wound healing, their role locally in burn wounds has not yet been studied. In *Chapter 4* we have investigated these acute phase proteins locally at a tissue level of a burn injury site.

II: Taphonomical and tissue degradation studies

Taphonomy is a relatively new and unknown science. It was first introduced in palaeontology and has since been embedded in the world of geo-archaeology and anthropology. Well known taphonomic features such as bone or cementum degradation, the development of livores, rigor mortis and calor mortis are all part of the decomposition process (9) of decaying organisms and are central to the study of taphonomy. Taphonomy can answer questions that are of interest to forensic pathologist such as ones

dealing with ongoing body decomposition. Large studies investigating human decomposition were performed in Knoxville at the anthropological research facility in the United States. However whether the obtained results can be extrapolated to conditions observed in The Netherlands is questionable. Therefore a study using a pig model, to address different typhonomic questions relating to body decomposition was undertaken and is presented in *Chapter 5* of this thesis.

III: Cardiovascular pathology

Acute myocardial infarction (AMI) is a leading cause of death in the western world (14, 15, 16) and needs to be ruled out in the first instance at an autopsy as the cause of death, also in forensic autopsies. AMI can be identified, as studies have shown, using routine histochemistry using the nitro blue tetrazolium staining method of heart slides. In this way AMI was able to be detected macroscopically at 3 hours or more after its onset (17, 18).

To detect cardiac infarctions earlier, studies looking at ultrastructural changes of the mitochondria in the heart have been described in both canine and rat hearts. Mitochondrial deposits which are small osmiophilic amorphous densities (19, 20) that can be detected using electron microsocopy as early as one hour post AMI (21) have been described. Other mitochondrial changes have also been reported to occur with AMI such as swelling of the mitochondria and the formation of disorganized cristae. However due to the fact that these changes are also observed during the autolysis process of cells (21), the ability to use electron microscopy to detect changes associated with early AMI in both canines and rat hearts has been questioned. Since autolytic changes also occur in human hearts, it is unclear whether mitochondrial deposits detected as early as half hour post mortem could be associated with AMI. In *Chapter 6* we investigated whether or not ultrastructural analysis of mitochodrial deposits can be used as a method for the detection of early AMI.

It is common knowledge that alterations to coronary artery structure can induce AMI by changing regional blood flow and provoking ischaemia (22). In an earlier study, the results obtained suggested that the induction of AMI can be aided by the presence of pre-existing aberrations in the intramyocardial microvascular, related to the induction of local inflammation in these arteries. Further ultrastructure studies were carried out to investigate whether or not intramyocardial capillaries also play a role in AMI induction. These studies are described in *Chapter 7* where the basement membrane thickness of intramyocardial capillaries was measured.

Another cause of ischemia and infarction that is widely seen in patients with other diseases is pulmonary embolism. As in the case of AMI, pulmonary embolism is a significant cause of morbidity and mortality (23, 24). An experimental model has shown that pulmonary embolism causes a very selective decrease in blood flow only to the right ventricular subendocardium (25) that might result in the reported increase in the level of CD68 positive macrophages in the right ventricles of patients with pulmonary embolism (26, 27). Although the study concluded that the influx of macrophages was a result of an ischemic episode to the heart that occurred subsequently to the pulmonary embolism, these ischemic changes were never proven. We wondered whether this could be explained by the induction of myocarditis in these patients. In *Chapter 8* of this thesis, the relationship between pulmonary embolism and (endo) myocarditis was thus investigated.

References

1. Oehmichen M, Schmidt V et al. Freisetzung von, Proteinase-Inhibitoren als vitale reaktion im fruhen post-traumatischen. *Intervall Z. Rechtsmed* 1989; 102: 461-472.

2. Dressler J. Bachmann L, Koch R et al. Enhanced expression of selectins in human skin wounds. *Int J Legal Med* 1998; 112: 39-44.

3. Rathmell JC, Thompson CB. The central effectors of cell death in the immune system. *Ann Rev Immunol* 1999; 17:781-828.

4. Lau SK, Chu PG, Weiss LM, CD163; A specific marker of macrophages in paraffin-embedded tissue samples. *Am J Clin Pathol* 2004; 122: 794-801.

5. Knight B. The Pathology of Wounds. In: Edward Arnold, editor. *Forensic Pathology.* 3 ed. London: 2004. P. 136-73.

6. Kanchan T, Menezes RG, Manipady S. Haemorrhoids leading to post-mortem bleeding artifact. *J. Clin Forensic Med* 2006 July; 13(5): 277-9.

7. Hammer U, Buttner A. Distinction between forensic evidence and post-mortem changes of the skin. *Forensic Sci Med Pathol* 2012 September, 8(3):330-3.

8. Dettmeyer R.B. Thrombosis and Embolism; Vitality, Injury age, Determination of skin wound age, and fracture age. In: Springer, editor. *Forensic Histopathology: Fundamentals and Perspectives.* 1 ed. Berlin: 2012. P.173-210.

9. DiMaio V. Time of Death-Decomposition. In: CRC Press, editor. *Handbook of Forensic Pathology.* 2 ed. London: 2007.

10. Dhennin C, Pinon G, Greco JM. Alterations of complement system following thermal injury: use in estimation of vital prognosis. *J Trauma* 1978; 18:129-33.

11. Barrett M. The clinical value of acute phase reactant measurements in thermal injury. *Scand J Plast Reconstr Surg Hand Surg* 1987; 21:293-5.

12. Oldham KT, Guice KS, Till GO, Ward PA. Activation of complement by hydroxyl radical in thermal injury. *Surgery* 1988; 104:272-9.

13. Thomas S, Wolf SE, Chinkes DL, Herndon DN. Recovery from the hepatic acute phase response in the severely burned and the effects of long-term growth hormone treatment. *Burns* 2004; 30:675-9.

14. de la Grandmaison GL. Is there progress in the autopsy diagnosis of sudden unexpected death in adults? *Forensic Sci Int* 2006;3:138-44.

15. Murray CJ, Lopez AD. Alternative projections of mortality and disability by cause 1990-2020: global burden of disease study. *Lancet* 1997; 349(9064):1498-504.

16. Murray CJ, Lopez AD. Mortality by cause for eight regions of the world: global burden of disease study. *Lancet* 1997; 349(9061):1269-76.

17. Nachlas MM, Schnitka TK. Macroscopic identification of early myocardial infarcts by alterations in dehydrogenase activity. *Am J Pathol* 1963;42:379-405.

18. Robbins SL, Cotran RS. Pathologic basis of disease. In: Kumar V, Abbas AK, Fausto N, editors. Disease of organ systems: the heart. *Philadelphia: Elsevier Sauders*, 2005;577-81.

19. Jennings RB, Baum JH, Herdson PB. Fine structural changes in myocardial ischemic injury. *Arch Pathol* 1965;79:135-43.

20. Jennings RB, Ganote CE. Structural changes in myocardium during acute ischemia. *Circ Res* 1974;35 (suppl 3):156-72.

21. United States, Canadian Academy of Pathology I. Cardiovascular pathology I. Cardiovascular pathology, clinicopathologic correlations and pathogenetic mechanisms, 37th edn. Philadelphia, PA: Williams & Wilkins, 1995.

22. Theroux P, Fuster V. Acute coronary syndromes: unstable angina and non-Q-wave myocardial infarction. *Circulation* 1998;97:1195-1206.

23. Douketic JD, Kearon C, Bates S, et al. Risk of fatal pulmonary embolism in patients with treated venous thromboembolism. *JAMA* 1998;279:458-62.

24. Goldhaber SZ, Visani L, De Rosa M. Acute pulmonary embolism: clinical outcomes in the international cooperative pulmonary embolism registry (ICOPER). *Lancet* 1999;353:1386-89.

25. Vlahakes GJ, Turley K, Hoffman JI. The pathophysiology offailure in acute right ventricular hypertension: hemodynamic and biochemical correlations. *Circulation* 1981;63:87-95.

26. Iwadate K, Tanno K, Doi M et al. Two cases of right ventricular ischemic injury due to massive pulmonary embolism. *Forensic Sci Int* 2001;116:189-95.

27. Iwadate K, Koi M, Tanno K, et al. Right ventricular damage due to pulmonary embolism: examination of the number of infiltrating macrophages. *Forensic Sci Int* 2003;134:147-53.