General discussion
DISCUSSION

Recent advances in medical technology but also changing political, ethical and financial views give rise to questions concerning the usefulness of clinical autopsies\(^1\). At the same time technological development improving the quality of both clinical and forensic autopsies is quite limited. There f.i. is a need to improve the determination of the cause of death, which is important in both clinical and forensic autopsies. While knowledge of wound age determination would not only have implications for autopsies in general, especially forensic autopsies\(^2\), but would also have enormous implications for wound age determination in living subjects by the forensic physician.

This we have studied in the present thesis.

I: Autopsy pathology

The decline of autopsies is a noted problem worldwide, f.i. the rate of consented autopsies in the U.K. was 0.7% in 2013 in comparison to 25% 30 years ago\(^3\). However, the autopsy is still regarded as the gold standard, being the most important tool for retrospective quality assessment of clinical diagnoses and its role as a key educational tool\(^4\). The reasons for this decline are variable; financial (non-reimbursement of autopsies), cultural (immigration), but most important is the limited autopsy requests of clinicians. For the latter different reasons can be pointed out. On one hand clinicians believe that with advances in laboratory tests and modern imaging techniques the autopsy had become redundant. On the other hand clinicians believe relatives will rarely consent to perform an autopsy, this is especially described in the U.K. after the Alder Hey Hospital incident\(^5\). However, research disputes this. For instance, in the division of lymphoid malignancies at St. Bartholomew’s hospital in London autopsy was suggested and discussed in depth with the relatives of all the patients who died in an eight-month period, between August 2009 and March 2010. Of the 18 families approached, 16 consented to autopsy, giving a consent rate of 89%\(^6\). This shows that when attention and time is given when asking for consent, the attitude of the general public is positive overall. We hypothesized that clinicians’ believe that with advances in medicine the autopsy has become superfluous, is unjust as well. In Chapter 2 we showed that when clinical diagnoses were compared with post-mortem diagnoses a major discrepancy rate of 23.5% was found. These consisted of 12.5% Goldman I cases, meaning that these diagnoses were related to the cause of death (COD) and that knowledge before death would have changed management of care and could have prolonged survival or cured the patient. The remaining 11% were Goldman II cases, meaning that these diagnoses were related to the COD, but that knowledge before death probably would not have changed the outcome\(^7,8\). These results are in line with recent literature\(^9-18\). However we now found a higher percentage of major discrepancies at autopsy compared with imaging findings during life which was mainly due to imaging of an
improper body part (e.g. CT-abdomen instead of CT-thorax) or with an improper imaging modality (e.g. X-thorax instead of CT-angio thorax). This disputes the belief of clinicians that due to advances in modern imaging techniques the autopsy had become redundant. This is in line with the findings of Combes et al., who found that percentages of major diagnostic discrepancies were similar between patients that had undergone modern diagnostic techniques and patients that had not. These findings thus emphasize the value of autopsies in our modern era. In Chapter 2 we also showed that microscopic examination has a major impact on macroscopic diagnoses made during clinical autopsy, namely it contributed to the final COD in 19.6% of cases, which is in accordance with previous studies. This confirms again the importance of performing microscopic examination in clinical autopsies.

In forensic autopsies the role of microscopic examination in identifying the COD is less clear and studies concerning the use of routine microscopic examination in forensic autopsies are contradictory, ranging from 0.5% to 8.4% of cases in which microscopic examination was necessary to establish the COD. A policy of histological sampling in all cases has financial and legal implications. Since there is a conflict in the U.K between the law and advise from regulatory bodies in relation to the sampling of tissue for microscopic examination during forensic autopsies, we investigated the role of microscopic examination in determining the COD at a forensic unit in England in Chapter 3. The Coroners Rules namely dictate that pathologists in England and Wales are authorized to retain only that material which ‘bears upon the COD’ or ‘the identification of the deceased’. In opposition to these rules, the general guidelines from The Royal College of Pathologists (RCPa) advise the pathologist to perform microscopic examination of all major organs in all cases. In the Netherlands this conflict however is non-existing, since there is no legislation regarding the retention of tissue during autopsy. We have now found that in 2% of our forensic cases microscopic examination was necessary to determine the COD, which is in line with previous studies showing that microscopic examination is required in only a small percentage of forensic cases to determine a COD. We therefore conclude that microscopic examination is not crucial in all forensic autopsies to provide a COD. We want to emphasize the importance of microscopic examination in clinical autopsies. In cases without a clear macroscopical COD, also in forensic autopsies, microscopic examination is always necessary, since certain natural causes of death can only be excluded microscopically, like f.i. (borderline-) lymphocytic myocarditis ((B-)LM), which we demonstrated in Chapter 4. To diagnose (B-)LM in endomyocardial biopsies (EMB) the so-called Dallas criteria were developed. According to these criteria, a myocarditis is diagnosed in case histologically inflammatory infiltrates are found within the myocardium associated with myocyte necrosis of non-ischemic origin. However through the years, these criteria have shown not to be that adequate, due to amongst others sampling error. Even more, criteria for the diagnosis of (B-)LM in autopsy hearts are not defined yet. In autopsy hearts there is a much lower risk of sampling error, provided that the myocardium is widely...
sampled. The Association for European Cardiovascular Pathology suggests sampling should be performed of the left and right ventricular anterior, posterior and lateral wall, the interventricular septum, the right ventricular outflow tract and a sample of both atria. However, both (epitopes of) lymphocytes and myocytolysis are not always discernable in H&E slides. For this we proposed a new method for diagnosing (B-)LM in autopsy hearts in Chapter 4, namely immunohistochemistry (LCA to identify B- and T-lymphocytes and C3d to visualize myocytolysis) in combination with the Dallas criteria (figure 1). We demonstrated that with these immunohistochemical stainings evaluated by the Dallas criteria 7.5% cases of (B-)LM were found, as opposed to 1.9% of cases when the Dallas criteria were applied on H&E stained myocardial slides only. Next to LCA and C3d staining we advise to perform MPO (visualizing neutrophilic granulocytes) and CD68 (visualizing macrophages) staining in cases with an unclear COD, to demonstrate or exclude a sepsis and/or stress (catecholamine-mediated) myocarditis, which f.i. can be found in case of respectively sepsis or pulmonary embolism. A proposed approach to analyze myocarditis in autopsy hearts is shown in figure 1.

II: Wound age determination

II-1: Wound age determination in autopsies

Wound age determination of skin injuries is important in forensic autopsies. The differentiation between vital and post mortem injuries remains challenging for the pathologist. Although macroscopical and histological characteristics have been proposed to diagnose wound vitality, these have not shown to be discriminative. Immunohistochemical markers related to inflammation have been studied extensively and proven to be helpful in wound age determination. Next to inflammation, coagulation is also induced in vital skin injuries. In Chapter 5 we tested our hypothesis that the expression of markers related to coagulation, namely fibronectin, CD62p and factor VIII can differentiate between early vital (up to 30 minutes old) and post mortem wounds. We indeed found a significant increase (p<0.01) in the expression of all three markers in wound hemorrhage in time. We have developed a probability scoring system of early skin wounds related to the expression levels of these markers in wound hemorrhage. By quantifying the staining, using an immunohistochemical score (IH score) from 0-3, a probability score for each marker was calculated at different time points (figure 2). The probability scores were 87%, 88% and 90% for respectively fibronectin, CD62p and factor VIII that a wound was non-vital in case an IH score of 0 (1: referring to figure 2). In case of an IH score of 1 or 2, the chances were 82/90%, 82/83% and 72/93% that a wound was a few minutes old (2). Finally, in case of an IH score of 3, the chances were 65%, 76% and 55% that a wound was 15-30 minutes old (3). For all three markers, an IH score 0 or 1 gave the probability of 0% that the wound was 15-30 minutes old (4). Moreover, in case of an IH score 3 the probability that the skin tissue was non-injured was 1%, 0%,
and 1% for fibronectin, CD62p and factor VIII, respectively (5). This system made it possible to differentiate between control samples, few minutes old injuries and 15-30 minutes old injuries. With this method a more solid and reliable estimation of wound age in early skin injuries in forensic autopsies is possible.

Figure 1: Diagnosis of myocarditis.
LCA, leukocyte common antigen; MPO, myeloperoxidase; LM, lymphocytic myocarditis; B-LM, borderline lymphocytic myocarditis.
Figure 2: Immunohistochemical analysis of early wounds in autopsies (step 4 of wound age determination).
IH score, immunohistochemical score: 1=minor, 2=moderate, 3=strong.
II-2: Wound age determination in living subjects (figure 3)

Not only in forensic autopsies but also in forensic medicine wound age determination is important, but then related to living subjects. In the Netherlands forensic physicians from the GGD are namely asked to give an estimation of the skin injury of a victim of violence. It however is known that the reproducibility of macroscopic findings is low and these findings will therefore not hold in court\textsuperscript{39,47-49}. Since it is not possible to excise skin wounds in living subjects, we wondered whether (immuno)histochemical analysis of skin biopsies, representing the border area of the wound, could improve the estimation of the age of a skin injury in living subjects. We collected skin biopsies of wounds of living subjects and subdivided them into 4 different timeframes (0.2-2 days, 2-4 days, 4-10 days and 10-25 days old). In Chapter 6 we studied morphological characteristics (ulceration, parakeratosis and hemorrhage) and extra-cellular matrix proteins and myofibroblasts (collagen III, -IV and α-SMA) in these skin biopsies. In Chapter 7 inflammatory cells, namely neutrophilic granulocytes (MPO), lymphocytes (CD45) and macrophages (CD68), and the inflammatory mediators MIP-1, IL-8, CML and vitronectin were studied. We finally generated a probability scoring system for all parameters. Four parameters (ulceration, vitronectin, MIP-1, IL-8) could differentiate between wounds younger and older than 10 days. Ulceration and vitronectin expression were only found in wounds of 10 days or younger; the probability that a skin injury was 0.2-10 days old in case of ulceration (1: referring to figure 3) or vitronectin (3) expression in the extravasate was therefore 100%. We found that in case >10 MIP-1 (Macrophage Inflammatory Protein-1) (4) or IL-8 (5) positive extravascular neutrophils and macrophages/mm\textsuperscript{2} were found the probability was 100% that a wound was 10 days old or younger. When parakeratosis (2) was present or >200 MPO (6) positive cells/mm\textsuperscript{2} were found the probability was 97% and 96% respectively that an injury was inflicted 10 days ago or less. The other markers (hemorrhage, collagen III, collagen IV, α-SMA, CD45, CD68, epidermal IL-8 expression and N(epsilon)-(carboxymethyl)lysine (CML) however were not discriminating as such. Notwithstanding this, for all of the above-mentioned markers a probability scoring system can be used to determine the likelihood of wound age more precisely (see table 1 of Chapter 6 and 7).
Figure 3: Immunohistochemical analysis of biopsies of wounds in living subjects (step 4 of wound age determination).
MIP-1, Macrophage Inflammatory Protein-1; IL-8, Interleukine-8; MPO, myeloperoxidase.
Worth of note, we found the highest number of neutrophils (1: referring to figure 4) and lymphocytes (2) in wounds up to 2 days old and of macrophages in wounds up to 4 days old (3)). This is consistent with the literature, where inflammatory cells are described to invade the site of injury within hours after wounding, achieving their maximum, depending on the type of inflammatory cell, several days post wound infliction\textsuperscript{2,60-64}. For the inflammatory markers MPO and CD68 significant differences between the age groups were found. For MPO a significant difference was found between the high number of MPO at 0.2-4 days (high) and the low number of MPO in 4-25 days old wounds. The same is true between 0.2-10 and 10-25 days old wounds. Also CD68 showed a significant difference between 0.2-4 (high number of CD68) and 4-25 days old wounds (lower number of CD68). However, CD45 showed a small, non-significant increase in wounds of 10 days or older. To the best of our knowledge, this is not described in the literature before. We want to emphasize that the biopsies reflect the border area of the injury and our studies are therefore not directly comparable to other studies in the field, since they mostly studied autopsy skin wounds.

These results show that (immuno)histochemistry can be used to improve the diagnosis of skin injuries in living subjects. The probability scoring system can be used to determine the individual likelihood of wound age for all markers.

\begin{figure}
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\includegraphics[width=\textwidth]{figure4.png}
\caption{Immunohistochemical analysis of inflammatory markers in biopsies of wounds in living subjects (step 4 of wound age determination\textsuperscript{2}).
MPO, myeloperoxidase.}
\end{figure}
CONCLUSIONS

In this thesis, we studied the value of clinical autopsies and additional techniques related to both clinical and forensic autopsies as well as forensic medicine. We showed that the belief that the autopsy had become redundant, because of modern laboratory and imaging techniques, is incorrect. We found a major discrepancy rate between clinical- and autopsy diagnoses of 23.5%, emphasizing the value of clinical autopsies in our modern era. We furthermore showed that microscopic examination is not crucial in all forensic autopsies; a finding that is of interest in countries where there is strict legislation regarding the retention of tissue at autopsy. However, (B-)LM is a diagnosis for which microscopic examination is required of the heart, and we propose a new method for diagnosing (B-)LM in autopsy hearts, namely combining CD45 and C3d stainings with the Dallas criteria.

Wound age determination is important in forensic autopsies but also in living subjects, related to forensic medicine. We produced a system with which it is possible to differentiate between control samples, few minutes old skin wounds and 15-30 minutes old skin wounds in autopsy material. With this method a more solid and reliable estimation of wound age in early skin injuries in forensic autopsies is possible. Furthermore, we showed that (immuno) histochemistry can be used to improve the diagnosis of skin injuries in living subjects. We produced a probability scoring system that can be used to determine the individual likelihood of wound age in skin biopsies of the border areas of the wounds in living subjects.
REFERENCES


