Polyvinylpyrrolidone deposition disease

The role of pathology in understanding disease and death in persons with opioid addiction and intravenous drug use

Ida Viken Stalund

Thesis for the degree of Philosophiae Doctor (PhD)
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Scientific environment

The candidate has been affiliated with the Department of Clinical Medicine (K1) at the University of Bergen and the Department of Pathology at Haukeland University Hospital. The Western Norway Health Region funded this project (Project number 912001).

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Ida Viken Stalund

Selected abbreviations

CKD	Chronic Kidney Disease
eGFR	Estimated Glomerular Volume
EM	Electron Microscopy
EMA	European Medicines Agency
EU	European Union
GI-tractus	Gastrointestinal tractus
H&E	Hematoxylin and eosin
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
IVDU	Intravenous drug use
kDa	Kilo Dalton
MW	Molecular weight
NOMA	Norwegian Medicines Agency
OSD	Opioid substitution drug
OST	Opioid substitution therapy
PAS	Periodic acid Schiff
PASM	Periodic acid methenamine silver stain
PRAC	Pharmacovigilance Risk Assessment Committee
PVP	Polyvinylpyrrolidone
TA	Tubular atrophy
WHO	World Health Organization

Abstract

Background and aims: The polymer polyvinylpyrrolidone (PVP) is commonly used as an excipient in drugs. Parenterally administered high molecular weight PVP cannot be excreted, and therefore accumulates in tissues, resulting in PVP deposition. In 2013, pathologists at Haukeland University Hospital discovered cases of PVP deposition in tissue samples from patients with opioid addiction and intravenous drug use (IVDU), most of whom were enrolled in the opioid substitution therapy (OST) program in Norway. Intravenous injection of a specific opioid substitution drug, an oral methadone syrup containing very high molecular weight PVP (PVP K90) was the suspected cause of PVP deposition in these patients. This discovery eventually led to the withdrawal of this methadone syrup from the market in Norway and the European Union (EU) and was the origin of the project on which this thesis is based. The main objective of this thesis was to study the pathological findings in patients with PVP deposition disease from injection of PVP-containing opioid substitution drugs and to assess the clinical consequences.

Methods: The principal methods used in the studies were thorough qualitative and quantitative evaluations of histological specimens collected for diagnostic purposes and at autopsies. The pathological findings were then correlated with clinical data.

Results: This thesis discusses the pathological and clinical findings of 33 patients with PVP deposition. All biopsies and autopsy samples revealed PVP deposits, and in some patients, PVP deposition was very extensive. The findings strongly indicate that PVP deposition has caused severe anemia, pathological fractures and chronic kidney disease. In two patients, PVP deposition likely caused the fatal outcome.

Conclusions: Intravenous use of an oral methadone syrup containing PVP K90 has led to PVP deposition in patients with opioid addiction and IVDU, in some patients likely resulting in severe disease and fatal outcome. This thesis underscores the importance of a thorough pathological investigation of tissue samples and shows how reporting potential adverse drug reactions, even by pathologists, can make a big difference for

patients. It also shows how well intended efforts to prevent unintended uses of drugs can have unwanted consequences.

Sammendrag

Bakgrunn og målsetting: Polymeren polyvinylpyrrolidon (PVP) er mye brukt som tilsetningsstoff i legemidler. PVP med høy molekylvekt kan ikke utskilles via nyrene og vil derfor opphopes i kroppens vev dersom det administreres parenteralt. I 2013 oppdaget patologer ved Haukeland universitetssykehus tilfeller av PVP-avleiring i vevsprøver fra pasienter med opioidavhengighet og intravenøs rusbruk. En av legemidlene som på den tiden ble brukt i legemiddelassistert rehabilitering (LAR) var en metadonsirup ment for oral bruk som inneholdt PVP med svært høy molekylvekt (PVP K90). Man mistenkte at intravenøs bruk av denne metadonsirupen var årsaken til PVP-avleiring hos pasientene. Denne oppdagelsen førte til at metadonsirupen ble trukket fra markedet i Norge og i EU, og var utgangspunktet for prosjektet som denne doktorgraden er basert på. Hovedmålsettingen for doktorgraden var å studere de patologiske funnene hos pasienter med PVP avleiringssykdom etter injeksjon av PVP-holdige legemidler for opioidsubstitusjon og å vurdere de kliniske konsekvensene.

Metoder: Studiene baserer seg i hovedsak på grundig kvalitativ og kvantitativ evaluering av vevsprøver tatt i diagnostisk øyemed og ved obduksjoner samt vurdering av de patologiske funnene opp mot kliniske data.

Resultater: Denne graden handler om de patologiske og kliniske funnene i 33 pasienter med PVP-avleiringssykdom. Alle biopsier og obduksjonsprøver viste PVP-avleiringer som i noen tilfeller var svært utbredt. Funnene tyder sterkt på at PVP-avleiring har forårsaket alvorlig anemi, patologiske beinbrudd og kronisk nyresykdom. Hos to pasienter forårsaket PVP-avleiringer sannsynligvis dødelig utfall.

Konklusjon: Intravenøs bruk av en metadonsirup som var ment for oral bruk, og som inneholdt PVP K90 som tilsetningsstoff, har forårsaket PVP-avleiringer hos ruspasienter. I noen tilfeller er det sannsynlig at dette har forårsaket alvorlig sykdom og død. Denne graden fremhever viktigheten av en grundig patologisk evaluering av vevsprøver og den viser hvordan melding om mulige bivirkninger, selv fra patologer, kan utgjøre en stor forskjell for pasientene. Den viser også hvordan velmente forsøk på å hindre uønsket bruk av medisiner kan få uønskede konsekvenser.

List of publications

Paper I:

Ida Viken Stalund, Gro Nygard Riise, Friedemann Leh, Tormod Karlsen Bjånes, Lars Riise, Einar Svarstad and Sabine Leh. Case Report: Polyvinylpyrrolidone deposition disease from repeated injection of opioid substitution drugs: report of a case with a fatal outcome. *F1000Research* 2021, 10:300, doi: 10.12688/f1000research.51927.2

Paper II:

Friedemann Leh*, **Ida Viken Stalund***, Tormod Karlsen Bjånes, Christian Ohldieck, Einar Svarstad and Sabine Leh. Polyvinylpyrrolidone deposition disease in patients with intravenous opioid use: a case series. *Human Pathology*, 2021 Jul 28;116:102-111. doi: 10.1016/j.humpath.2021.07.009

*Equal contributions

Paper III:

Ida V. Stalund, Heidi Grønseth, Finn P. Reinholt, Einar Svarstad, Hans-Peter Marti and Sabine Leh. Chronic Kidney Disease from Polyvinylpyrrolidone Deposition in Persons with Intravenous Drug Use. *CJASN* April 2022, 17 (4) 518-526; doi: 10.2215/CJN.13681021

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1 Introduction

In 2013, pathologists at Haukeland University Hospital discovered a puzzling finding in several biopsies. The biopsies revealed infiltrates of macrophages with an extended, "bubbly" cytoplasm with peculiar staining characteristics. These bubbles (vacuoles) stained light blue with hematoxylin and eosin (H&E), faint or bright red with Congo red and grey or black with periodic acid methenamine silver (PASM). By a coincidence, they also became aware of an autopsy performed two years earlier that showed the same findings. Eventually, they recognized these findings as deposits of polyvinylpyrrolidone (PVP) (1). PVP deposition had previously been reported as an adverse effect of parenteral administration of PVP-based plasma expanders and extended-release medications (2). However, this application of PVP was discontinued several decades ago (3). Therefore, the origin of these deposits was a puzzle. However, these three patients had one thing in common: a history of opioid addiction and intravenous drug use (IVDU), and all were receiving opioid substitution therapy (OST). This fact led the pathologists to suspect opioid substitution drugs (OSDs) as a possible source of PVP in these patients. More of the story behind the project can be read in Appendix 1.

1.1 Opioid addiction and intravenous drug use

1.1.1 Definitions and epidemiology

Opioids are a group of chemically related drugs that exert their pharmacological effects by interacting with opioid receptors (4). This group of drugs includes both prescription drugs for severe pain, such as morphine and oxycodone, and illicit drugs, such as heroin. Opioid receptors are involved in the regulation of many physiological functions, reflected in the effects and side-effects of opioid drugs (4). While the effect on pain processing is the main effect sought for in the clinical setting, the effect on the brain's reward system, inducing euphoria and drowsiness, is central to the development of opioid addiction. Opioid addiction is defined as a compulsion to seek intake of opioid drugs and a loss of control in limiting drug intake (5).

Opioid addiction is a far-reaching problem in Norway and the rest of the world. In recent years, several countries have experienced a rapid increase in problematic opioid use (6). This so-called opioid epidemic has become a major public health crisis with serious consequences for individuals, families and whole communities. In Norway, the prevalence of opioid addiction is estimated to be 2.0-2.4 per 1000 (7, 8). The typical route of opioid administration (oral ingestion, smoking, intravenous or subcutaneous injection, etc.) varies from country to country. In Norway, intravenous injection is the most common route of administration, and the prevalence of persons with IVDU in Norway is among the highest in Europe (7, 8).

1.1.2 Complications from opioid addiction and IVDU

The complications associated with opioid addiction and IVDU are diverse and depend on the extent of drug use and the type of drug administered. Complications can affect both physical and mental health, as well as socio-economic factors. Some of the complications most relevant to this thesis are briefly presented in the following sections.

Persons with opioid addiction and IVDU are at increased risk of multiple diseases and premature death (9). The annual crude mortality rate in this group is 2% per year, 15 times higher than in the age- and sex-matched general population, although data vary widely between countries (10). The increased morbidity and mortality in this population is only partly related to the drugs themselves. Associated diseases and lifestyle factors related to opioid addiction and IVDU also contribute (11). The most common causes of death are drug overdose, associated diseases such as blood-borne infections and violent deaths (accidents, homicide and suicide) (10-12).

Fatal and non-fatal drug overdoses

Nonfatal and fatal overdoses represent a major concern in the care of persons with opioid addiction and a major public health problem (13). Although prevalence varies between populations, there has been a trend towards increasing overdose death rates and overdose-related hospitalizations in most countries in recent decades (13). A Norwegian cohort study published in 2008 found an overdose-specific mortality rate of 2.4 per 100 person-years among persons with untreated opioid addiction (14). The

overdose-specific mortality rate in Norway is among the highest in Europe (7, 8). The lifetime risk of experiencing a non-fatal overdose is much higher, ranging from 20% to 70% in different reports (15). Although non-fatal, such incidents can cause severe morbidity due to hypoxia, aspiration, rhabdomyolysis and other adverse outcomes (16, 17).

Chronic viral infections

Due to the use of contaminated equipment, such as syringes and needles, there is a high prevalence of blood-borne viral infections among persons with IVDU (18). Worldwide, it is estimated that 18% of persons with IVDU are infected with human immunodeficiency virus (HIV), 52% are hepatitis C virus (HCV)-antibody positive, and 9% are hepatitis B virus (HBV) surface antigen positive (19). However, there is considerable variation in prevalence between geographical regions and countries. In Norway, the prevalence of HIV-infection (1.3 %) and active HBV infection (1 %) among people with IVDU is much lower than the estimated prevalence worldwide (20). However, the prevalence of chronic HCV-infection (43%) is comparable to the global prevalence (20).

If left untreated, 10-20% of persons with chronic HCV-infection will progress to cirrhosis within 20 years, with a high risk of developing liver failure and hepatocellular carcinoma (21). In addition, chronic HCV-infection is associated with several extrahepatic disease manifestations, such as mixed cryoglobulinemia, chronic kidney disease (CKD), and type 2 diabetes mellitus (22). In 2016, the World Health Organization (WHO) set a goal to eliminate viral hepatitis as a public health threat by 2030 (23). To achieve this goal, efforts must be made to reduce the number of new infections and to make diagnostic tests and treatment more widely available (23). Although there are differences between geographical regions of the world, the work to achieve this goal is slowly progressing. (24, 25). In Norway, the goal is set to reduce the prevalence of HCV infection by 90% by 2023 (26). In pursuit of this goal, Norway is among the countries in Europe with the highest coverage of needle and syringe programmes (25), and the number of municipalities with syringe exchange programmes is gradually increasing (27). The HCV-treatment coverage is also

increasing, although not yet at the pace required to reach the goals set by WHO and the Norwegian Ministry of Health and Care Services (28).

Non-viral infections

Local skin and soft tissue infections are common among people with IVDU and are a major source of morbidity (29-31). Such infections are associated with the use of non-sterile equipment and intentional or unintentional subcutaneous injections (30, 32). Histopathological findings include phlebitis, abscess formation and necrotizing fasciitis (29). Such local infections can lead to sepsis, infective endocarditis and bacterial embolization to internal organs (33). Furthermore, the chronic inflammation resulting from repeated or chronic skin- and soft tissue infections puts the individual at risk of developing AA amyloidosis, which is discussed further below (34).

Injection of oral medications, such as crushed tablets, is also common among people with IVDU (35, 36). Injection of oral medications as well, as the use of inappropriate solvents, introduces foreign material, such as talc and cellulose derivatives, into the circulation (36). Foreign material, often birefringent, is therefore a frequent finding in biopsies and autopsies in this patient group (37). Such foreign material can cause embolization in internal organs, leading to hypoperfusion and granulomatous reactions in the surrounding tissue (36, 38, 39).

1.1.3 Kidney function and kidney disease

The kidney is one of the target organs of several diseases associated with IVDU and pathological findings in the kidney are a major part of this thesis. For this reason, the normal function and histology of the kidney and kidney disease in persons with IVDU will be discussed in more detail in the following sections.

Kidney function and histology

The kidney is composed of multiple nephrons with a surrounding interstitium and a supplying vascular system (Figure 1a and b). A single nephron consists of a glomerulus located in the renal cortex and a tubule that passes through both the renal cortex and the renal medulla (Figure 1b) (40).

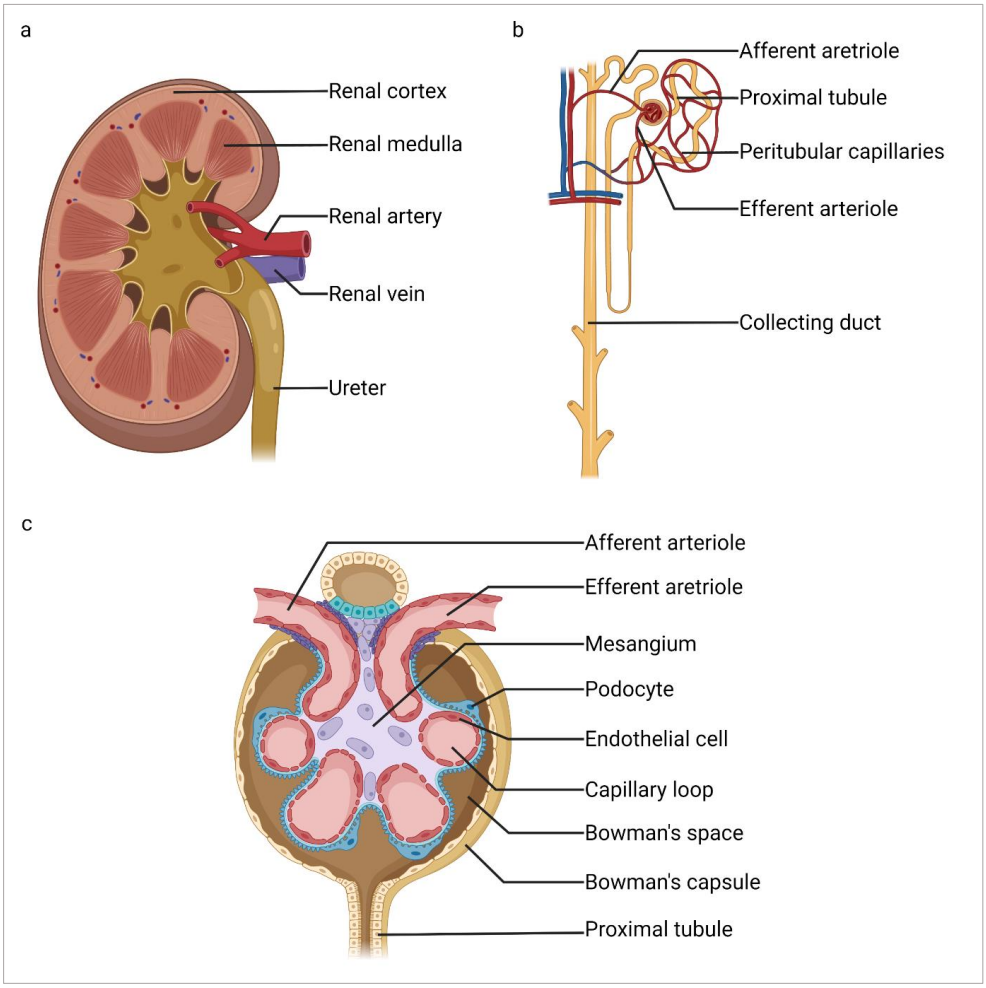

Figure 1: Kidney anatomy and function. *a: The kidney consists of multiple lobules, each containing both renal cortex and medulla, and the collecting system, which pours into the ureter. Each kidney is supplied by a single renal artery and drained by the renal vein. b: The cortex and medulla contain multiple nephrons. Each nephron consists of a glomerulus and a tubule. The latter can be subdivided into the proximal and distal tubule, which have distinct functions. c: The glomerulus is a spherical collection of interconnected capillaries supported by the mesangium which contains mesangial cells and mesangial matrix. The outer aspect of each capillary loop is covered by podocytes. The Bowman's capsule surrounds the capillary loops. The space between the capillary loops and the Bowman's capsule, the Bowman's space is continuous with the tubule. Created with BioRender.com*

The glomerulus is a specialized bundle of capillaries where passive filtration of the blood takes place (Figure 1c). The filtrate pours into the tubule, a long and winding tube lined by tubular epithelium, where active reabsorption and excretion takes place. The blood supply to each glomerulus is provided by the afferent arteriole, which leads the blood into the glomerulus. The blood leaves the glomerulus through the efferent

arteriole which subsequently branches into a capillary network that supplies both the cortex and the medulla. Between the nephrons is the interstitium, which is comprised of cells, extracellular matrix, and interstitial fluid. All compartments, the glomerular, the tubulointerstitial and the vascular compartment, are active components of the kidney parenchyma important for maintaining the homeostasis of kidney function. The compartments are codependent, and disease affecting one will impact the function of the others. While diseases primarily affecting the glomerulus typically present clinically with proteinuria or hematuria, diseases primarily affecting the tubulointerstitium present with kidney insufficiency (increased serum-creatinine and reduced glomerular filtration rate (GFR)).

The evaluation of kidney tissue samples involves the assessment of pathological changes in the glomerular, the tubulointerstitial and the vascular compartments (Figure 2a) (40). A normal glomerulus (Figure 1c and 2b) shows an expanded capillary tuft with thin capillary walls and a limited number of cells in the mesangium between the capillaries. The filtration of the blood to form the primary urine occurs through the capillary wall, which consists of the following three layers: endothelium, basement membrane and podocytes. The podocytes form finger-like processes (foot processes) that cover the basement membrane. The diaphragm between the podocyte foot processes is the least permeable layer of the filtration barrier. The space surrounding the capillary tuft (Bowman's space) is narrow and lined by flattened epithelial cells, and the surrounding capsule (Bowman's capsule) is slender.

The cortical tubulointerstitial compartment (Figure 2c) shows back-to-back proximal tubules, distal tubules and collecting ducts. The proximal tubules are particularly prominent and lined by tall, cuboidal tubular epithelial cells with an apical brush border. Each tubule is surrounded by a thin basement membrane. The narrow interstitium between the tubules contains few cells and peritubular capillaries.

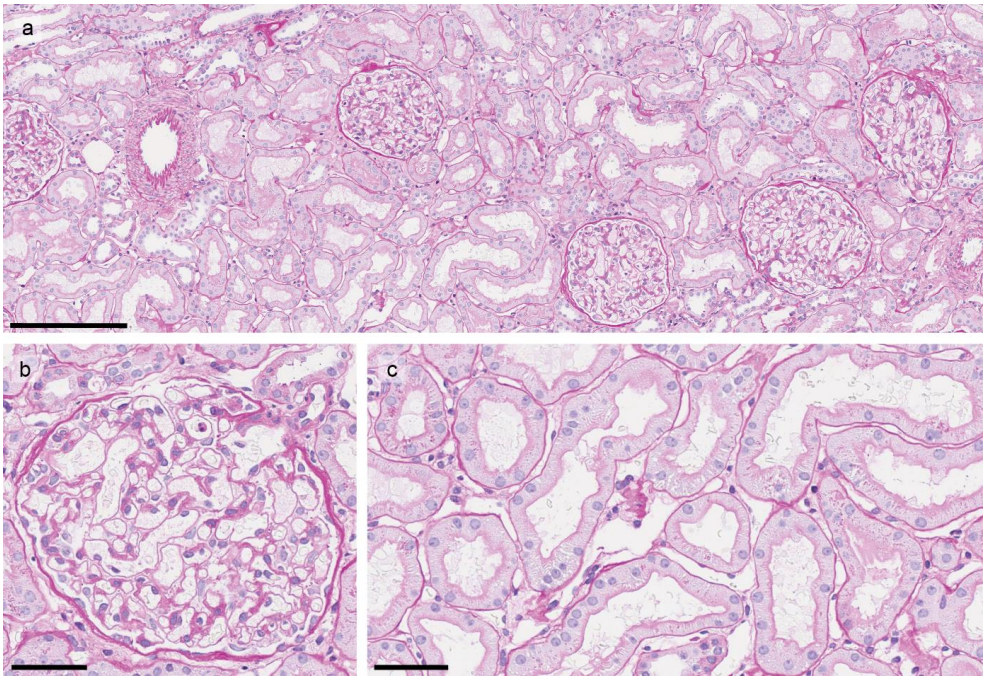

Figure 2: Normal histology of the renal cortex (PAS). *a: Overview showing sections through glomeruli, proximal and occasional distal tubules and an artery. Scale bar 50 µm. b: Normal glomerulus. The capillary tuft is expanded and surrounded by a slender Bowman's capsule. Capillary walls are thin and the mesangium shows scarce mesangial matrix and low cellularity. In the upper right corner, a normal arteriole (probably efferent) is visible. Scale bar 50 µm. c: Normal proximal tubules laying almost back-to-back, separated by interstitium with low cellularity and scarce fibrotic tissue. Scale bar 50 µm.*

The vascular compartment contains the blood vessels (artery visible in Figure 2a) that supply and drain the kidney tissue. The vasculature ranges from large arcuate arteries to afferent and efferent arterioles to the capillaries which drain into venules and subsequently into larger veins. The arteries and arterioles, which are the most prominent vessels in tissue sections, have an even wall with a thickness proportional to the size of the vessel.

Kidney disease in persons with opioid addiction and IVDU

Persons with opioid addiction and IVDU have an increased risk of acute and CKD (41). In addition, persons in this patient group who develop end-stage kidney disease have poor outcomes on renal replacement therapy and higher mortality compared to patients without drug dependency (42, 43). Kidney disease related to opioid addiction and IVDU can affect all compartments of the kidney, causing both glomerular,

tubulointerstitial and vascular disease (44, 45). The following sections will focus on kidney disease associated with opioid addiction and IVDU. However, poly-drug use is common in this patient group (46) and common findings related to some other drugs will also be mentioned.

Glomerulopathies

In the 1970s, several publications described kidney disease with proteinuria associated with heroin use in the USA (47, 48). This led to the suspicion that heroin had a direct nephrotoxic effect and the term heroin-associated nephropathy arose (48). The most common findings were focal segmental glomerulosclerosis and membranoproliferative glomerulonephritis (47). Despite a rise in heroin use, the incidence of heroin-associated nephropathy has decreased since the early 1990s. At the same time, the incidence of HIV and HCV-infection and associated glomerulopathies has increased. This development has challenged the theory that glomerulopathies in heroin users are caused by a direct nephrotoxic effect of heroin (45). It is now thought that most cases of glomerulopathies in heroin users are related to the viral (e.g. HCV-infection) and bacterial (e.g. endocarditis) infections associated with IVDU (47).

Several histological types of glomerulopathies are associated with HCV-infection. The most commonly reported are membranoproliferative glomerulonephritis (Figure 3a) and mesangial proliferative glomerulonephritis, but other patterns of glomerulonephritis have also been reported (49, 50). Common to most of these patterns is deposits of immune complexes in the glomerulus. Immune complex deposits are also evident in infection-related glomerulonephritis, which can develop during or after a bacterial infection (51). Infection-related glomerulonephritis in patients with IVDU is commonly related to staphylococcal skin and soft tissue infections and to endocarditis (51). The glomerular patterns seen are variable, but a diffuse exudative hypercellular glomerulonephritis with prominent endocapillary hypercellularity is often present (51).

HIV-infection is also associated with a variety of kidney disease entities (52). HIV-associated nephropathy was the first entity of HIV-associated kidney diseases to be described. It is a consequence of local HIV-infection of the kidney and the pattern seen

is a collapsing form of focal segmental glomerulosclerosis (Figure 3b) (52). As HIV-associated nephropathy is mainly seen in people of African descent, it is rarely seen in Norway (52). Other kidney diseases associated with HIV-infection are immune complex glomerulopathies and thrombotic microangiopathy (52).

In recent years, there have been several reported cases of thrombotic microangiopathy and acute kidney injury associated with intravenous use of oral formulations of the opioid analgesic oxymorphone (45). Thrombotic microangiopathy is a reaction pattern resulting from a range of disease entities with a common pathogenesis of endothelial damage leading to arteriolar and capillary thrombosis (53). Kidney involvement is common and the primary morphological changes are microthrombi in capillaries and arterioles and evidence of endothelial damage (54). Typical clinical symptoms include hemolytic anemia, thrombocytopenia and kidney insufficiency.

Tubulointerstial disease

In the setting of opioid addiction, acute tubular necrosis is the most common histopathological pattern of acute kidney disease (45, 55). Acute tubular necrosis is characterized by tubular epithelial injury with tubular dilation, loss of the brush border and eventually epithelial cell necrosis and shedding of cells into the tubular lumen. This pattern of injury is seen in both toxic and ischemic injury. Among persons with opioid addiction, acute tubular necrosis is commonly associated with myoglobinuria due to rhabdomyolysis after prolonged immobilization (Figure 3c) (55).

Furthermore, interstitial inflammation was common in an autopsy study on kidney findings in deceased persons suspected of illicit drug use (56). However, inflammation sufficient to diagnose chronic interstitial nephritis was found in only one person. The same study reported that interstitial fibrosis and tubular atrophy in >10 % of the tissue sample area was found in 16 % of the deceased.

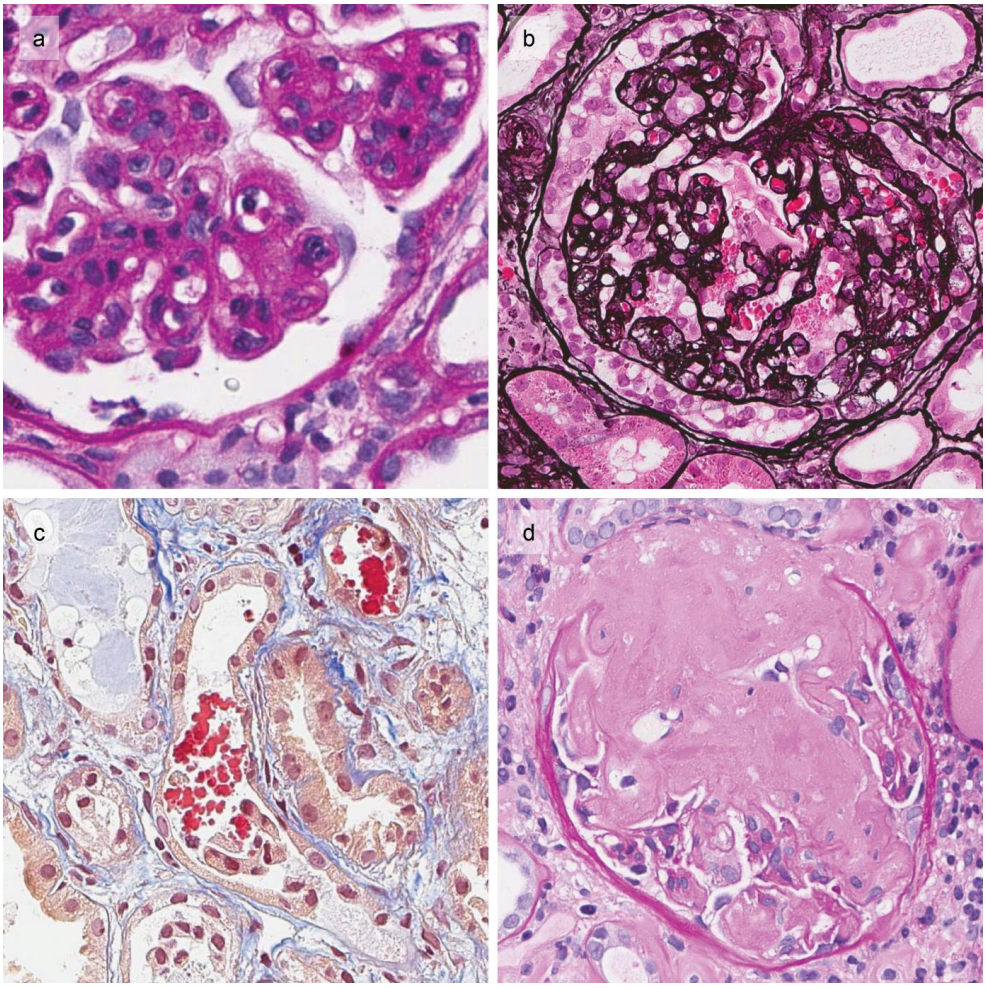

Figure 3: Examples of pathological findings seen in kidney biopsies from patients with opioid addiction and/or injecting drug use. *a: Membranoproliferative glomerulonephritis (PAS). Micrograph showing mesangial hypercellularity and thickened peripheral capillary walls with focal double contours. B: Collapsing FSGS (HIVAN) (PASM). The capillary tuft is collapsed and there is proliferation of podocytes in Bowman's space, which also contain protein droplets. C: Rhabdomyolysis-induced acute tubular necrosis (trichrome). Tubuli show varying degree of flattened epithelium. In the lumen, there are myoglobin casts and detached viable epithelial cells. D: Amyloidosis (PAS). The glomerulus shows extensive deposits of amorphous, acellular material which is amyloid.*

Vascular disease

The above cited autopsy study by Buettner et al. found that arteriosclerosis (52 %), arteriolosclerosis (28 %) and signs of glomerular ischemia (42 %) in the kidney tissue were common findings (56). Arteriosclerosis, signs of glomerular ischemia and a

diagnosis of hypertensive-ischemic nephropathy were associated with a positive test for cocaine. This association has been shown in other studies as well, and the proposed mechanism of this association is the vasoconstrictive effect of cocaine (45).

AA amyloidosis

AA amyloidosis is a complication of chronic infection and inflammation. It is a condition characterized by extracellular deposition of misfolded proteins, specifically the acute-phase reactant amyloid A (57). The high prevalence of skin and soft tissue infections among people with IVDU puts this population at risk of developing AA amyloidosis (58). Kidney disease with proteinuria and renal insufficiency is often the first disease manifestation of AA amyloidosis, and the kidneys tend to be the most severely affected organ (57). Amyloid deposits appear as pale eosinophilic, amorphous material in the H&E stain and weakly positive material in the periodic acid-Schiff (PAS) stain (Figure 3d). The deposits stain with Congo red and appear apple-green under polarized light (59). In kidney biopsies, amyloid deposits can be seen in the glomerular mesangium and in glomerular capillary walls, arterial and arteriolar walls, and in the tubulointerstitium (59). Deposition of amyloid fibrils in the glomerular capillary walls damages the filtration barrier, resulting in severe proteinuria or a nephrotic syndrome. Renal AA amyloidosis is a common histopathological diagnosis in kidney biopsies from patients with IVDU and several reports in the last decade postulate an increasing prevalence of AA amyloidosis in this patient group (34, 60-62).

1.2 Opioid substitution therapy

1.2.1 History

The concept of OST was introduced in Canada and USA in the 1960s (63). The rationale for OST was to prevent injection-related disease and intoxication by prescribing oral long-acting opioids, such as methadone, as a substitute for injectable short-acting heroin (63). The HIV-epidemic and an increase in deaths from opioid overdoses in the 1990s, led to the introduction of OST in Norway in 1998 (64, 65). At that time, the program was dimensioned for approximately 700 patients nationally (65). Full opioid detoxification was mandatory for enrollment in an OST program (64).

Since then, the requirement for full detoxification has been relaxed and the number of enrolled patients has steadily increased. At the end of 2020, more than 8000 patients were receiving OST in Norway (66).

According to the regulations for OST in Norway, the purpose of OST is to improve the quality of life of people with opioid addiction and to help individuals to improve their optimal level of coping and functioning. The aim is also to reduce the harm caused by opioid addiction and the risk of overdose deaths (67).

These regulations are supported by studies showing that OST reduces mortality among patients with opioid addiction, and OST is now considered the most effective harm-reducing measure in this patient group (14, 68, 69). A Norwegian prospective cross-registry study on mortality among patients who applied for OST between 1997 and 2003 found a significant reduction in the all-cause mortality risk in the intention-to-treat population compared with the pre-treatment population (14). This tendency was even stronger for overdose-specific mortality. In addition to reducing mortality, studies have shown that OST has positive effects on health and socioeconomic factors such as employment and crime involvement (69). Recognizing the importance of OST, the WHO included methadone and buprenorphine in the Model List of Essential Medicines in 2005 (70, 71).

1.2.2 Medications and distribution

The medications used in OST are slow-release oral opioids. They are prescribed as a fixed daily dose that is individually adjusted for each client. The slow absorption and metabolism of these medications results in limited symptoms of intoxication and a longer-lasting opioid effect, thereby limiting abstinence symptoms and drug wanting (64).

The Norwegian OST-program uses three different medications (64, 72):

- Methadone – a full opioid agonist, usually formulated as a syrup.
- Buprenorphine – a partial opioid agonist, most commonly formulated as a sublingual tablet.
- A combination of buprenorphine and naloxone, most commonly formulated as a sublingual tablet or film. The opioid antagonist naloxone, which has little pharmacological effect with oral or sublingual administration, causes withdrawal symptoms when the drug is administered parenterally.

The main rule for the distribution of OSDs is daily, supervised intake. However, daily attendance can be an obstacle to the rehabilitation to a normal daily life, which is one of the main goals of the treatment. Therefore, patients who demonstrate social and treatment stability may be given take-home doses for up to one week at a time (64).

Despite the extensive documentation of the positive effects of OST, approximately 10% of patients with opioid addiction do not benefit sufficiently from the treatment (73). For this group of patients, heroin assisted treatment is an alternative (74). Heroin assisted treatment involves daily distribution of short-acting opioids, such as diacetylmorphine (heroin), morphine or hydromorphone for injection or oral ingestion under supervision (73). Eight countries, mostly in Europe, have provided heroin-assisted treatment as a part of OST for several years (73). A review of six randomized controlled trials found that patients in heroin-assisted treatment had a reduction in illicit heroin use and greater retention in treatment compared with patients in traditional OST (75). Norway launched a heroin-assisted treatment program in Oslo and Bergen in 2022, which is being scientifically monitored and evaluated as a trial project (76).

1.2.3 Challenges in OST

There is little controversy about the benefits of OST. However, there are two main concerns related to OST: diversion and injection of OSDs. Both pose a potential health risk in terms of fatal and non-fatal overdoses and both undermine the legitimacy of OST (77, 78).

Diversion is defined as the leakage of OSDs to out-of-treatment persons, as OST clients share their prescribed OSDs with friends or sell them on the illicit market. This is quite common: A Norwegian study on OSD use among out-of-treatment persons with opioid addiction and IVDU, found that more than one fourth had used OSDs in the previous four weeks (77). Another Norwegian study investigated methadone-related deaths and found that approximately 80% of the deceased persons were not enrolled in the OST-program (79). It is likely that the methadone taken by the deceased persons was diverted from the OST-program. It is thought that daily, supervised intake at least partly counteracts the problem of diversion, whereas allowing take-home doses increases the risk of diversion.

Another issue of concern is injection of OSDs. OSDs are medications intended for oral administration. However, injection of OSDs is relatively common among persons with opioid addiction and IVDU, both in and out-of-treatment (35, 77, 80). Reported motivations for injecting the medication include rapid onset of effect, enhanced drug effect, greater euphoria and "needle fixation" (80, 81). As described in section 1.1.3, injection of any oral medication can lead to both local and systemic complications (36, 82). Over the years, attempts have been made to prevent injection of OSDs. Measures include increasing viscosity by adding thickeners to methadone syrups (81), reducing the attractiveness of injection by adding an opioid antagonist to buprenorphine formulations (35), and prescribing drug formulations that dissolve more rapidly when administered sublingually (83). Despite these measures, injection still occurs (35, 83).

1.3 Polyvinylpyrrolidone

Polyvinylpyrrolidone (PVP, also known as povidone) is a water-soluble polymer of N-vinyl-2-pyrrolidone units (Figure 2). It was developed in Germany in the 1930's by Walter Reppe, professor in chemistry (3). PVP has many favorable properties such as universal solubility, film forming, adhesiveness and resistance to thermal degradation. As a result, it has applications in many modern industries, including the pharmaceutical, food and beverage and cosmetics industries (3).

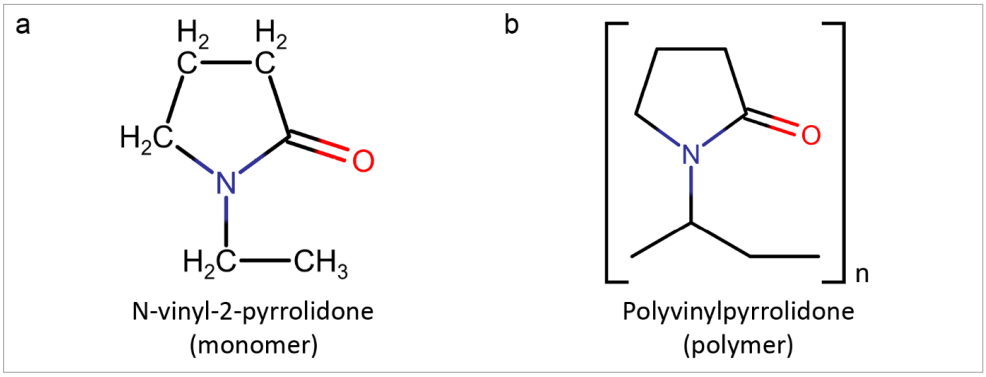

Figure 4: The molecular structure PVP. *Molecular structure of the monomer N-vinyl-2-pyrrolidone (a) and the polymer polyvinylpyrrolidone (b).*

As PVP is a polymer, it can be produced with any number of N-vinyl-2-pyrrolidone units resulting in PVP products with different molecular weights (MW) (84, 85). The different PVP products are assigned nominal K-values based on the viscosity of an aqueous solution of the product, which in turn depends on the MW range of the PVP molecules it contains (Table 1). The different PVP products can be broadly classified as low (K12 and K17), moderate (K25 and K30) and high MW (K90) (84). However, the low and moderate MW products contain a proportion of high MW PVP molecules, and vice versa (84).

Table 1: Different PVP-products and their molecular weight (MW) range.

PVP variant	Molecular weight range (kDa)
PVP K12	2 – 3
PVP K17	7 – 11
PVP K25	28 – 34
PVP K30	44 – 54
PVP K90	1 000 – 1 500

1.3.1 Uses in the pharmaceutical industry

Because of PVP's many useful properties (Table 2), it is a common excipient in pharmaceuticals. There are also several trials on novel drug delivery systems using PVP (85). The main applications of PVP in the pharmaceutical industry are shown in Table 2.

Table 2: Main applications of PVP in the pharmaceutical industry.

Function	Pharmaceutical form
Binder	Tablets, capsules, granules
Bioavailability enhancer	Tablets, capsules, granules, pellets, suppositories, transdermal systems
Film former	Ophthalmic solutions, tablet cores, medical plastics
Solubilizer	Oral, parenteral and topical solutions
Taste making	Oral solutions, chewing tablets
Lyophilisation agent	Injectables, oral lyophilisates
Suspension stabilizer	Suspensions, instant granules, dry syrups
Hydrophilizer	Medical plastics, sustained release forms, suspensions
Adhesion	Transdermal systems, adhesive gels
Stabilizer	Enzymes in diagnostics
Intermediate	Povidone-iodine as active ingredient
Toxicity reduction	Injectables, oral preparations
Thickener	Oral drops, eye drops, solutions and syrups

Reprinted by permission from Springer Nature Customer Service Centre GmbH: Springer Verlag Polyvinylpyrrolidone Excipients for Pharmaceuticals: Povidone, Crospovidone and Copovidone by Volker Bühler (2005) (84).

1.3.2 Pharmacokinetic properties

Absorption of orally administered PVP is minimal. Close to 100% of orally administered PVP can be recovered in the feces, and most is recovered in the first 24 hours after administration (86, 87). When PVP is administered parenterally, there is almost no metabolic degradation of the molecules (88). The predominant route of elimination is via glomerular filtration and urinary excretion (88-90). The rate and extent of renal excretion of PVP depends on the MW of the molecules (3, 88-90). While PVP with MW less than 25 kDa (K12 and K17) is readily excreted, PVP with MW up to 120 kDa (e.g. K25 and K30) takes days, weeks or months to eliminate depending on the MW. Different values of MW have been reported in the literature as

the upper limit for glomerular filtration of PVP: 70, 94 and 116 kDa (3). However, there is a consensus in the literature that PVP with MW above 120 kDa (e.g. K90) is too large for glomerular filtration, and will therefore be retained in the body if injected.

It is important to bear in mind that the different PVP products consist of molecules with a range of MWs. In his synopsis of PVP excipients for pharmaceuticals, Bühler illustrates the MW distribution of a PVP K30 product, which contains a considerable amount of higher MW PVP molecules (Figure 5) (84).

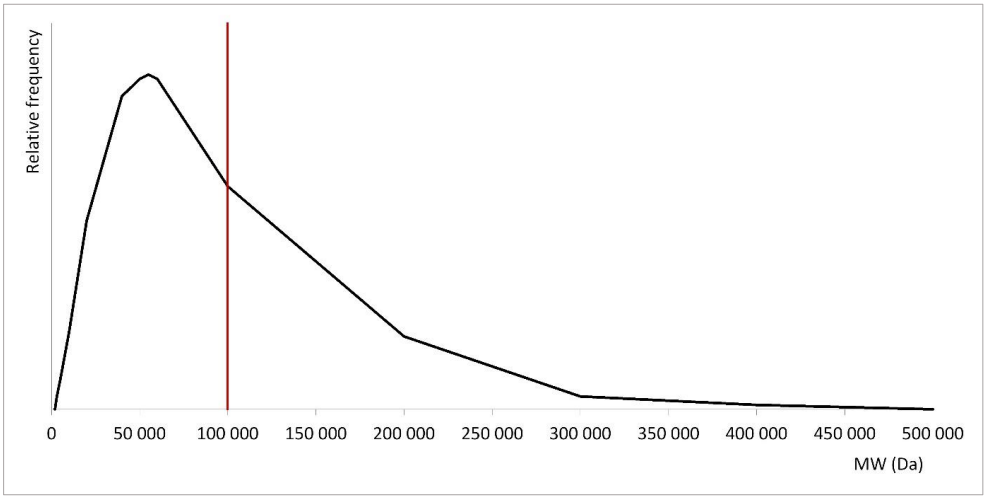

Figure 5: The typical molecular weight distribution curve of a PVP K30 product. *The vertical red line visualises an assumed upper limit for glomerular filtration. Modified after Bühler (84).*

Due to the relative and absolute impermeability of the glomerular filtration barrier to moderate and high MW PVP, only PVP products with a K-value below 18 are recommended for parenteral use.

1.3.3 Historical use of PVP in pharmaceuticals and PVP deposition

After its development in 1938, solutions of PVP were widely used as plasma expanders to treat blood loss in Germany during the Second World War. Several variants of PVP-based plasma expanders were used with mean MWs ranging from 11 to 50 kDa (2). The use of PVP as a plasma expander continued until the 1960s when it was replaced by other alternatives (2). After its introduction, several other applications of PVP were developed. It was used intravenously for the evaluation of both enteral

protein loss and renal clearance (2). Due to PVP's ability to bind toxins, attempts have been made to use it in the treatment of neonatal icterus (91), diphtheria and tetanus (2). Of particular interest to this thesis is the use of moderate MW PVP as a retardant in injectable extended-release medications for hormone substitution (2).

The first report of PVP accumulation in tissue was published by Bargmann in 1946 (92, 93). He described vacuolated cells, foam cells, in the spleen of rats and dogs after intravenous infusion of the PVP-containing plasma expander Periston, and postulated that the vacuoles contained PVP. Subsequently, a number of case reports, case series and experimental studies were published investigating the elimination, retention and storage of PVP in the body after infusion of plasma expanders (88, 94-101).

Studies on the pharmacokinetics of PVP

Several experimental studies, both in animals and humans, have been published on the distribution, metabolism and elimination of intravenously administered PVP. One of the most comprehensive studies on this subject was published by Ravin, Seligman and Fine in 1952 (88). The authors infused rats, rabbits, dogs and humans with radiolabeled PVP of various MWs to evaluate the retention of PVP in the circulation, the urinary and biliary excretion, the metabolism and the distribution of retained PVP in different organs and tissues. They found that PVP was neither metabolized nor excreted in the bile. Furthermore, that both the retention of PVP in the circulation and its excretion in the urine depend on the MW range of the infused PVP product. The majority of the low MW fractions were cleared from the circulation within six hours and excreted in the urine during the first 48 hours. The results were reversed for the high molecular weight fractions (PVP K50); 75% of the infused PVP was still in circulation at 12 hours and less than 20% was excreted in the urine during the first 48 hours. Based on radioactivity measurements of the different organs of autopsied individuals, they found that the organs of the reticuloendothelial system (liver, spleen, bone marrow and lymph nodes) showed the highest concentration of deposited PVP. However, the authors detected radioactivity in all organs (central nervous system not reported).

PVP deposition from the use of PVP-based plasma expanders

After the initial description of PVP deposition by Bargmann, other studies followed that investigated PVP deposition from the use of PVP-based plasma expanders. Traenckner (1954) reported on 300 adults, describing storage in the spleen, bone marrow, kidney and liver up to 3 years after infusion of a plasma expander containing PVP with a MW range of 20 – 80 kDa (101). Gall et al. (1953) reported findings in serial liver biopsies from 25 individuals obtained up to 13 months after receiving a single infusion of 1000 ml of 3.5 or 4.5% PVP (MW range 20 – 80 KDa) (96). They described basophilic deposits within Kupffer cells or free in the sinusoids. Deposits were more frequent and abundant with time, indicating a redistribution of the deposits over time. A follow-up study on the same 25 individuals by Altemeier et al. (1954) reported autopsy findings in seven patients who died of unrelated causes up to 19 months after the infusion (94). Autopsies revealed vacuolated macrophages containing PVP in the spleen, lymph nodes, liver, bone marrow, and adrenal cortex of all seven patients. PVP deposits in the intestinal mucosa, heart, lungs, atherosclerotic plaques of the aorta, pancreas, kidney, bladder, prostate, gallbladder, esophagus and thyroid gland were more inconsistent findings. The authors postulated that the deposition in these organs was caused by chemotaxis of the PVP-containing macrophages due to inflammatory processes.

In 1966 and 1967, two publications described the findings from autopsy studies on deceased patients who had received PVP-containing plasma expanders as part of the treatment for surgical or medical conditions (97),(98) . Honda et al. described an increasing incidence and extent of PVP deposition with increasing doses of PVP (97). The patients receiving the highest dose of PVP (>140 grams) showed PVP deposition in all organs examined and experienced complications from the same organ systems. The authors suggested a causal relationship between PVP deposition and complications. Kojima, Takahashi and Honda also observed a correlation between the dose of PVP and the extent of PVP deposition. In one patient, a young woman being treated for cervical cancer, they considered PVP deposition to be the cause of death. She died of acute liver failure shortly after receiving daily plasma expander infusions

for two months. The autopsy revealed extensive PVP-deposition in the liver and marked dissociation of the liver cells.

With the exception of these two autopsy studies, most authors reported only transient, minor or no functional impairment of organs affected by PVP-deposition from the use of PVP-based plasma expanders.

PVP deposition from the use of injectable extended-release medication

From the 1950s to the late 1970s, moderate to high MW PVP was used as a retarding agent in medications for intramuscular and subcutaneous injection. Most of these were hormone substitution drugs, in particular antidiuretic hormone for the treatment of diabetes insipidus. Unlike plasma expanders, which were usually administered a few times over a short period of time, these medications required daily injections for several years. There are case reports describing clinically relevant PVP-deposition from the use of such medications (102-106). The reported patients had received daily injections for up to 19 years (103), with a total amount of PVP administered of up to 3 kg (107). The most frequently reported clinical findings are cutaneous lesions in the form of a papular rash (107, 108) or pseudotumorous lesions at the injection site (102, 109, 110). Case reports also described PVP deposition in bone and bone marrow, causing spontaneous fractures and anemia (102, 106). Other findings included secondary amenorrhea associated with ovarian deposition (103), respiratory insufficiency associated with lung parenchyma deposition (110), polyneuropathy associated with peri- and intraneural deposition (102) and renal insufficiency associated with renal deposition (105, 106). Edelmann et al. also described a case of severe upper gastrointestinal obstruction and hydronephrosis due to retroperitoneal masses with PVP deposition (103).

PVP deposition from the use of PVP for nutritional support and as a detoxifying agent

In most countries of the world, the use of moderate and high MW PVP in preparations for parenteral administration ended in the late 1970s. However, there are some more recent Taiwanese case reports showing continued use of PVP-based preparations for nutritional support and as a “blood tonic” (111-115). The most commonly reported

findings in these cases were skin lesions associated with dermal PVP deposition (112, 113), anemia and/or other cytopenias associated with extensive PVP deposition in the bone marrow (112, 114), and spontaneous fractures and bone destruction associated with osseous deposition (112-115).

1.4 The use of PVP in opioid substitution drugs

In 2013, when the first cases of PVP-deposition were recognized, five different OSDs were marketed in Norway. Three of them (two different formulations of buprenorphine sublingual tablets and one methadone oral solution) contained PVP as an excipient (Table 3).

Table 3: PVP-containing OSDs marketed in Norway in 2013. Type of PVP product, relative and absolute amount of PVP in typical user dose.

	Buprenorphine tablet	**Methadone syrup**
Active substance	8 mg Buprenorphine	2 mg/mL Methadone
PVP[1] type (MW[2])	K30 (44 – 54 kDa[3])	K90 (1000 – 1500 kDa)
PVP per tablet/solution	8 mg	11.7 mg/mL
PVP in typical user dose	8 mg	585 mg (50 mL)

Type of PVP product, relative and absolute amount of PVP in typical user dose.
[1]Polyvinylpyrrolidone; [2]Molecular weight; [3]kiloDalton.

One buprenorphine tablet contains approximately 8 mg of PVP K30. The buprenorphine tablets containing PVP K30 have been marketed since 2000, and still are (last assessment 14.11.2022).

The methadone oral solution contained 11.7 mg of PVP K90 per ml of solution, added as a viscosity enhancer with the aim of reducing the risk of injection (116). The methadone formulation was originally developed for the Norwegian market due to particular requirements among OST clients in Norway. It received marketing authorization in Norway in 2006 and in Sweden, Denmark, Finland, United Kingdom and Malta in 2009 (117). Based on sales statistics, the PVP-containing methadone was

popular among OST clients in Norway. Between 2008 and 2012, this formulation accounted for approximately 30% of the methadone syrup sold in Norway (Figure 6) (118). Market shares for the PVP-containing methadone syrup were particularly high in western Norway, at around 50% between 2009 and 2011.

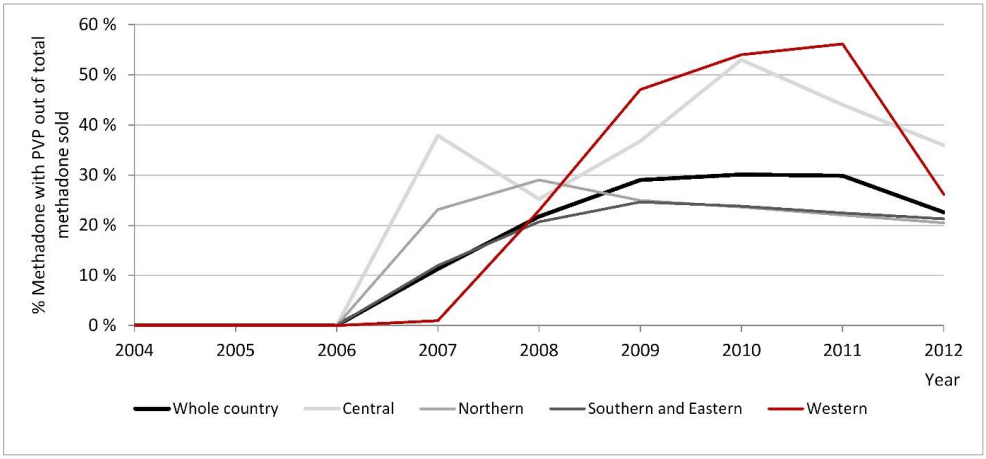

Figure 6: Market share of the methadone syrup containing PVP K90. *Proportion of the total volume of methadone (DDD) sold containing PVP K90 in Norway, nationally and in each health region in 2004-2012. Source: Norwegian Institute of Public Health. Wholesaler-based drug statistics (date of data extraction: Feb 25th 2014) (118)*

1.4.1 PVP-deposition in patients with opioid addiction and IVDU

The first three cases of PVP deposition in tissue samples from patients with opioid addiction and IVDU were diagnosed at Haukeland University Hospital in 2013. When pathologists became aware of this phenomenon, more cases were discovered. The evidence for a connection between the PVP deposition and OSDs was strong enough to justify reporting the findings to the Norwegian Medicines Agency (NOMA) as possible adverse effects in August 2013 (116). NOMA considered it possible or probable that the findings were related to the injection of PVP. Consequently, they reported the cases to the European Medicines Agency (EMA) on 2 April 2014 and suspended all methadone oral solutions containing PVP from the Norwegian market pending further investigations (116). The referral to EMA triggered the Pharmacovigilance Risk Assessment Committee (PRAC) to review the benefit-risk balance of oral methadone formulations containing PVP. The PRAC concluded that

the benefits for the oral methadone solution containing povidone K90 did not outweigh the risks. As a result, the methadone formulation containing PVP K90 was suspended from the European market as of July 10, 2014 (116).

2 Aim of study

The overall aim was to study the pathological findings and clinical picture in patients with PVP deposition disease following injection of PVP-containing opioid substitution drugs.

Specific aims:

1. To assess the likelihood that PVP deposition was caused by injection of the methadone formulation containing PVP K90.
2. To assess the extent of PVP-deposition in individual patients.
3. To assess the adverse effects caused by PVP deposition in general and in the kidneys in particular, using clinicopathological correlation.

3 Methods

The main methods used in the studies were thorough qualitative and quantitative evaluation of histological specimens collected in a diagnostic setting. All assessments were performed on digital slides scanned at 40x magnification. Clinical data, in terms of laboratory findings and journal information on the clinical course of the patients, were collected and correlated with the histopathological findings.

3.1 Formal approvals

The patient who was the subject of the first publication gave his consent for publication before he died. The patient's closest relative read the manuscript and gave consent prior to publication.

The Regional Committee of Medical and Health Research Ethics approved the data collection and experiments for the second and third publication (REK 27687, 2013/1925) and approved the exemption from the consent requirement for the use of information and biological material collected in health-care services.

The list of included patients was checked against the Registry of Withdrawal from Biological Research Consent (last assessment 16.04.2021).

All studies were conducted in adherence to the Declaration of Helsinki.

3.2 Materials

The collection of cases began in 2013 and was performed continuously as newly received biopsies with PVP deposition were recognized at the Department of Pathology, Haukeland University Hospital. If previous biopsies from individuals were available in our archive, these were re-evaluated. In general, the department receives biopsies from the former Hordaland County. However, non-neoplastic kidney biopsies are also sent from the entire Western Norway Health Region and other parts of the country (catchment areas of Nordland Hospital Trust, Telemark Hospital Trust, Østfold Hospital Trust and Hospital of Southern Norway). Twenty-three cases were

included after routine diagnostic evaluation at the Department of Pathology, Haukeland University Hospital. In addition, 10 non-neoplastic kidney biopsies were included from Oslo University Hospital (Rikshospitalet) and St. Olav's University Hospital. We did not perform a systematic search for cases in the departments' archives.

All microscopic evaluation was performed on digital slides. Slides were scanned with Scanscope XT (Aperio Technologies, Vista, California) at 40x magnification resulting in a resolution of 0.25 μm per pixel. We used ImageScope viewing software (version 12, Aperio Technologies, Vista, California) to view and annotate digital slides and to make measurements. Digital slides were stored and administrated as a dedicated research project in the eSlide Manager database (Aperio Technologies, Vista, California). Unless new stains and immunohistochemical analyses were required, the evaluation was performed on the original tissue slides.

For patients from the Western Norway Health Region (18 patients), clinical data were collected from digital patient records. For patients from other health regions (15 patients), clinical data were collected from pathology referral forms, the Norwegian Renal Registry and by retrieval of specified data from local patient records.

All tissues and data evaluated in our studies were gathered as part of the treatment or follow-up of patients in the health-care setting. No patient appointments or collection of laboratory- and tissue samples were performed for the purpose of this study.

3.3 Detection of PVP deposits

PVP deposits (macrophages with PVP-containing vacuoles) are most often easily recognized by the characteristic light blue color of the vacuoles in H&E-stained tissue (Figure 7a). To further verify that the content of the vacuoles was indeed PVP, most biopsies were also stained with Congo red (vacuoles stain red) and/or PASM (vacuoles stain grey or black) (Figure 7b and c). In some biopsies, immunohistochemical examination with CD 68 was performed in the diagnostic setting or by us to verify the histiocytic origin of the PVP-containing cells (Figure 7d).

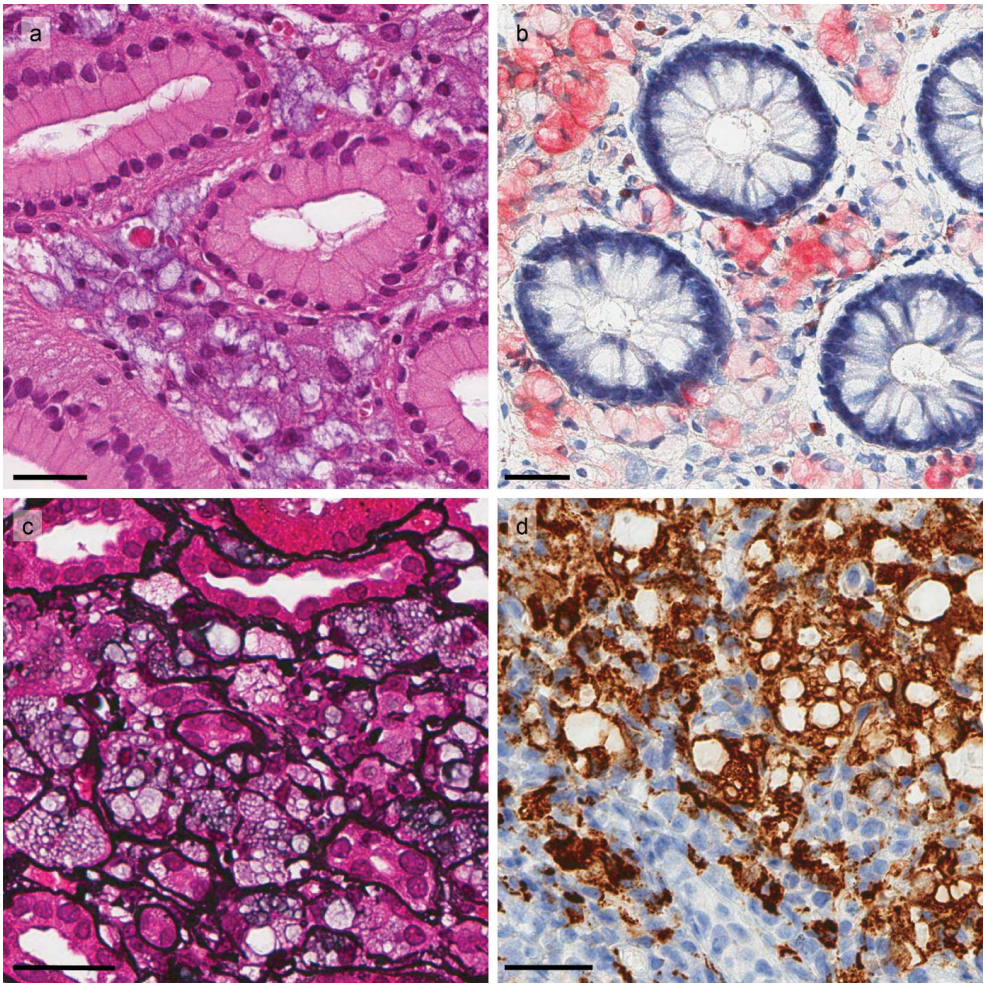

Figure 7: Staining properties of PVP deposits. *The PVP-containing vacuoles stain light blue or grey in H&E (a), bright or faint red in Congo red (b) and grey or black in PASM (c). The vacuolated cells are CD68-positive macrophages (d). All scale bars 30 µm.*

3.4 Calculation of total body PVP load

One of our aims was to assess the likelihood that the observed PVP deposition was in fact caused by injection of the methadone formulation containing PVP K90. To accomplish this, we performed calculations of the amount of PVP deposited in an individual, the total body PVP load. These calculations were based on our own histological findings and previously published data:

Bone marrow biopsies from two patients showed almost complete occupation of the bone marrow space by PVP-containing macrophages. To simplify calculations, we assumed that the entire bone marrow mass of these patients was replaced by deposited PVP.

The average weight of the bone marrow in adult males is approximately 3000 g according to a study by Mechanik published in 1926 and summarized by Woodard and Holodny in 1960 (119, 120).

By use of spectrophotometric analysis of a subcutaneous nodule with extensive PVP deposition, Reske-Nielsen et al found that the amount of PVP in PVP-saturated tissue was 12 mg PVP/ g of dry weight tissue (102). In order to use this finding, we had to convert it to 0,005 g PVP/ g of "wet" tissue.

A study on the distribution of retained PVP in human organs and tissues two months after infusion of C^{14}-labeled PVP (K33) found that 6.5 % of the retained PVP was situated in the bone marrow (88).

On the basis of these variables, we were able to estimate the total body PVP load in the two patients (calculations presented in Table 4, paragraph 4.1.5).

We performed further calculations to estimate the number of injected doses of the buprenorphine tablet containing PVP K30 and the methadone syrup containing PVP K90 required to induce the observed level of PVP retention. The manufacturers of the buprenorphine and methadone formulations containing PVP informed us of the absolute amount of PVP in a typical dose of each drug (Table 3 in paragraph 1.4). Based on previous studies on the excretion of PVP, we assumed that 100 % of injected PVP K90 would be retained (3). The proportion of injected PVP K30 that would be retained is more uncertain. There are several studies on the excretion and retention of moderate MW PVP, and the results vary. (3, 88). Based on the results of these studies, we assumed that 50 % of injected PVP K30 would be retained.

3.5 Quantitative methods performed on kidney biopsies

All kidney biopsies had previously been evaluated in a routine diagnostic setting which includes a series of special stains (PAS, PASM, Congo red, trichrome) and immunohistochemical examinations (immunoglobulins IgA, IgM, IgG, and complement factors C1q and C3). As part of the study, we re-evaluated all biopsies and performed semi-quantitative assessments of findings in the glomerular, tubulointerstitial and vascular compartments. To allow further statistical evaluation, we performed quantitative assessments of key findings: the extent of tubular atrophy, the extent of PVP deposition and the glomerular volume.

3.5.1 Area fraction of tubular atrophy

We quantified the biopsy area fraction showing tubular atrophy using the point counting method on PAS-stained digital slides (121, 122). We defined an atrophic tubule as a tubule with a thickened basement membrane and/or a reduction in tubule diameter. Digital slides were extracted from Aperio ImageScope image viewing software in JPG format with the number of pixels of both edge lengths reduced to 80%. The extracted images were imported into the open source software ImageJ (123). A grid with an area per point of 100 000 pixel^2 (edge length approximately 100 μm) was randomly placed on the image. Three categories of points (defined as the intersection of the two arms of the cross) were counted: points hitting atrophic tubulointerstitial areas, points hitting non-atrophic tubulointerstitial areas, and points hitting other structures such as large vessels and perivascular fibrous tissue or glomeruli. The medulla was excluded from the evaluation. The percentage of the cortical tubulointerstitial area affected by tubular atrophy was calculated as the number of points hitting atrophic tubulointerstitial areas divided by the total number of points hitting the cortical tubulointerstitium.

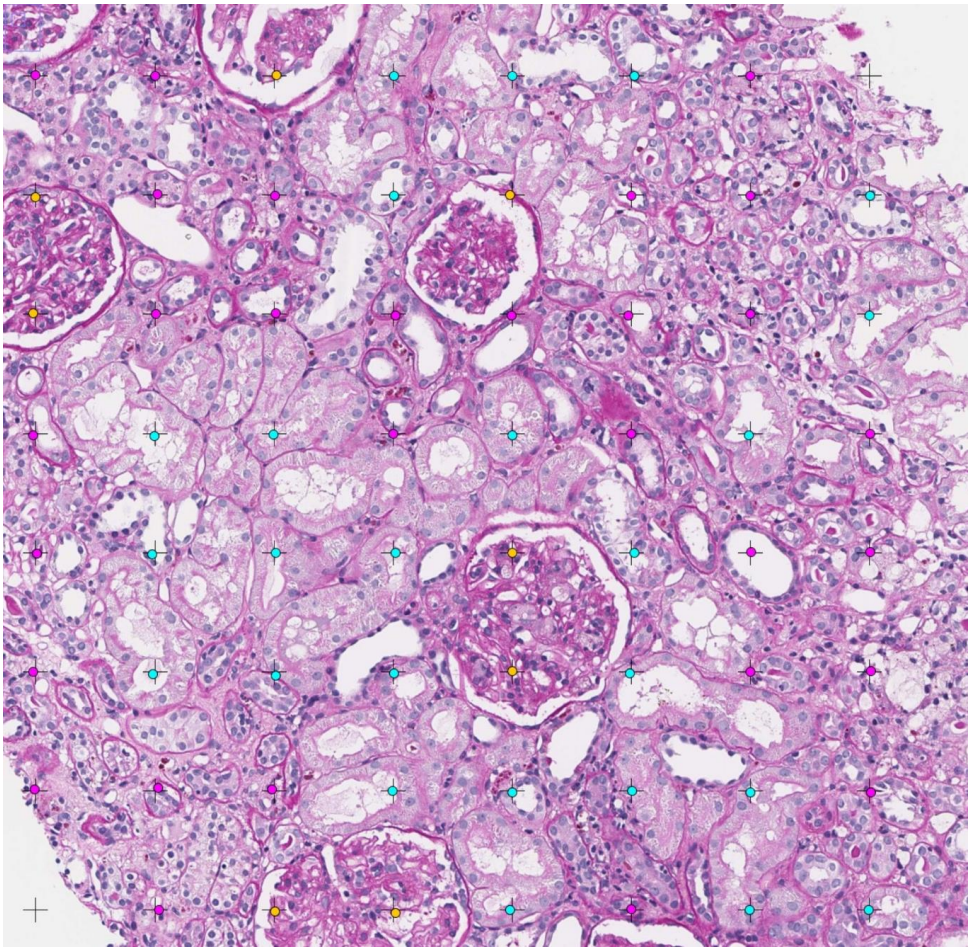

Figure 8: Point counting method. Screenshot from ImageJ. Purple crosses mark atrophic tubulointerstitial areas, blue crosses mark non-atrophic tubulointerstitial areas and yellow crosses mark other structures.

The principle of the method is illustrated in Figure 8. The number of points hitting areas with tubular atrophy is 29 (purple), the number of points hitting areas without tubular atrophy is 25 (blue). The area fraction of tubular atrophy is:

$$A_A(TA, tubulointerstitium) = \frac{29}{29 + 25} \times 100 = 54\%$$

To be certain that a grid with an area per point of 100 000 pixel^2 provided a reliable estimate of the area fraction of tubular atrophy, a pilot study was performed in which 5

repeated measurements were made with randomly placed grids (areas per point 100 000 pixel^2 and 25 000 pixel^2). The coefficient of error (CE) was then calculated:

$$CE(\overline{A_A}) = \frac{s}{\sqrt{n} \times \overline{A_A}}$$

where $\overline{A_A}$ is the mean area fraction of tubular atrophy, n the number of observations and s the standard deviation (121). The coefficient of error for these measurements was 0.03 for the percentage of tubular atrophy for both grid sizes thus supporting the assumption that a grid with an area per point of 100 000 pixel^2 would result in an acceptable coefficient of error and that a denser grid would not significantly improve the coefficient of error.

3.5.2 The extent of PVP deposition

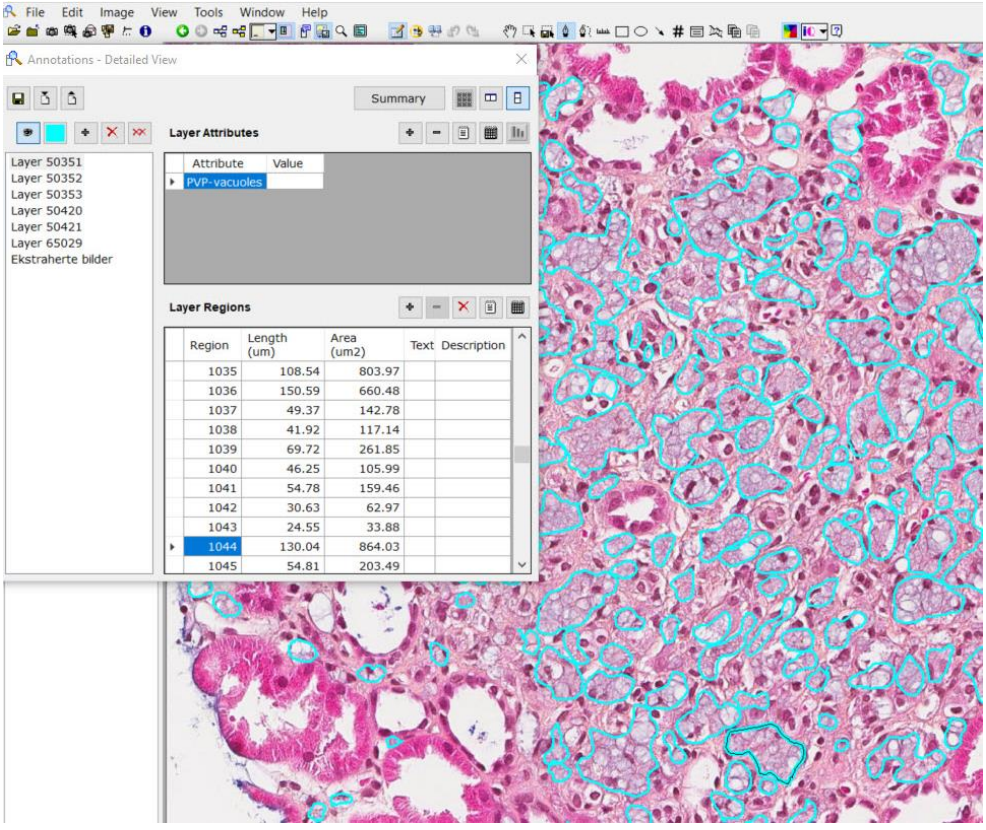

Figure 9: Area measurement of PVP deposits. *Screenshot from Aperio ImageScope. Individual areas of PVP-containing vacuoles are encircled. The areas encircled are automatically calculated.*

To quantify the extent of PVP deposition in each biopsy, we measured the area occupied by PVP-containing vacuoles by encircling the vacuoles with the pen tool in the Aperio ImageScope image viewing software. The sum of all individual areas gave the total area occupied by PVP-containing vacuoles (Figure 9). We also measured the total biopsy area using the pen tool. As some biopsies consisted mainly of medulla, the medulla was included in the measurement. Dividing the total area occupied by vacuoles by the total biopsy area gave the area fraction occupied by PVP-containing vacuoles.

3.5.3 Glomerular volume

To calculate individual glomerular volumes and the mean glomerular volume in each biopsy, we applied the stereological method of Weibel and Gomez (124, 125). The Weibel-Gomez formula estimates the volume of a structure from a single cross-section area (A), the shape of the structure (β – shape coefficient) and a size distribution factor (κ – size coefficient). For the estimation of the volume of a glomerulus (V_{glom}), a spherical shape (β = 1.38) and a size coefficient of 1.01 are assumed:

$$V_{glom} = \frac{{A_{glom}}^{1.5} * 1.38}{1.01}$$

The shape coefficient for a sphere adjusts for the fact that some glomeruli are sectioned through the periphery of the capillary tuft, giving a smaller cross-sectional area than if they were sectioned through the center. The size distribution coefficient adjusts for the fact that glomeruli vary in size, and larger glomeruli are statistically more likely to be sectioned. We obtained the glomerular cross-section areas (A_{glom}) by encircling the glomerular tufts with the annotation pen tool in ImageScope.

3.6 Electron microscopy (EM) methods

PVP-containing vacuoles in the glomeruli were visible by light microscopy. However, it was not possible to determine whether they were located in the mesangium, endothelium or in podocytes. This information is important because finding PVP-containing vacuoles in podocytes would indicate that PVP-molecules had been

filtered. This in turn would mean that PVP K90 can be excreted by glomerular filtration **or** that the PVP deposited has a lower MW than we assumed. With the aim of detecting PVP-containing vacuoles in mesangial cells, endothelial cells and podocytes, we re-evaluated electron microscopy (EM) specimens or, if this was not possible, we examined EM images taken at the time of primary evaluation.

The presence of vacuolated cells in glomeruli is common, especially podocytes often contain vacuoles. In particular, lipid-filled vacuoles can be difficult to distinguish from PVP-containing vacuoles based on the ultrastructural appearance (126). To facilitate differentiating PVP-containing vacuoles from lipid-filled vacuoles, we developed a set of criteria. We based these criteria on previous literature on the ultrastructural appearance of PVP deposits (105, 127), comparisons between light microscopic findings in toluidine blue-stained sections and corresponding electron microscopic images, and on comparisons between the ultrastructural appearance of vacuoles in known PVP cases and vacuoles in kidney biopsy samples from patients without PVP exposure.

To classify vacuoles as PVP vacuoles we defined the following criteria:

1. At least three round/oval vacuoles of similar size in a single cell.
2. Vacuole content has low to moderate electron density and low-grade granularity.
3. Vacuole profile diameter 0.3 – 1.5 µm.
4. Vacuoles are not collocated with osmiophilic material, which would be suspicious for lipofuscin.

3.7 Statistical methods

Statistical analyses of quantitative morphometric data and clinical data were performed using SPSS for Windows, version 24. In Paper III, we tested for statistically significant correlations using the Spearman's rank correlation coefficient for non-parametric data. Statistical significance was assumed at a P-value < 0.05.

4 Summary of results

An overview of the biopsy and autopsy findings is shown in Figure 10.

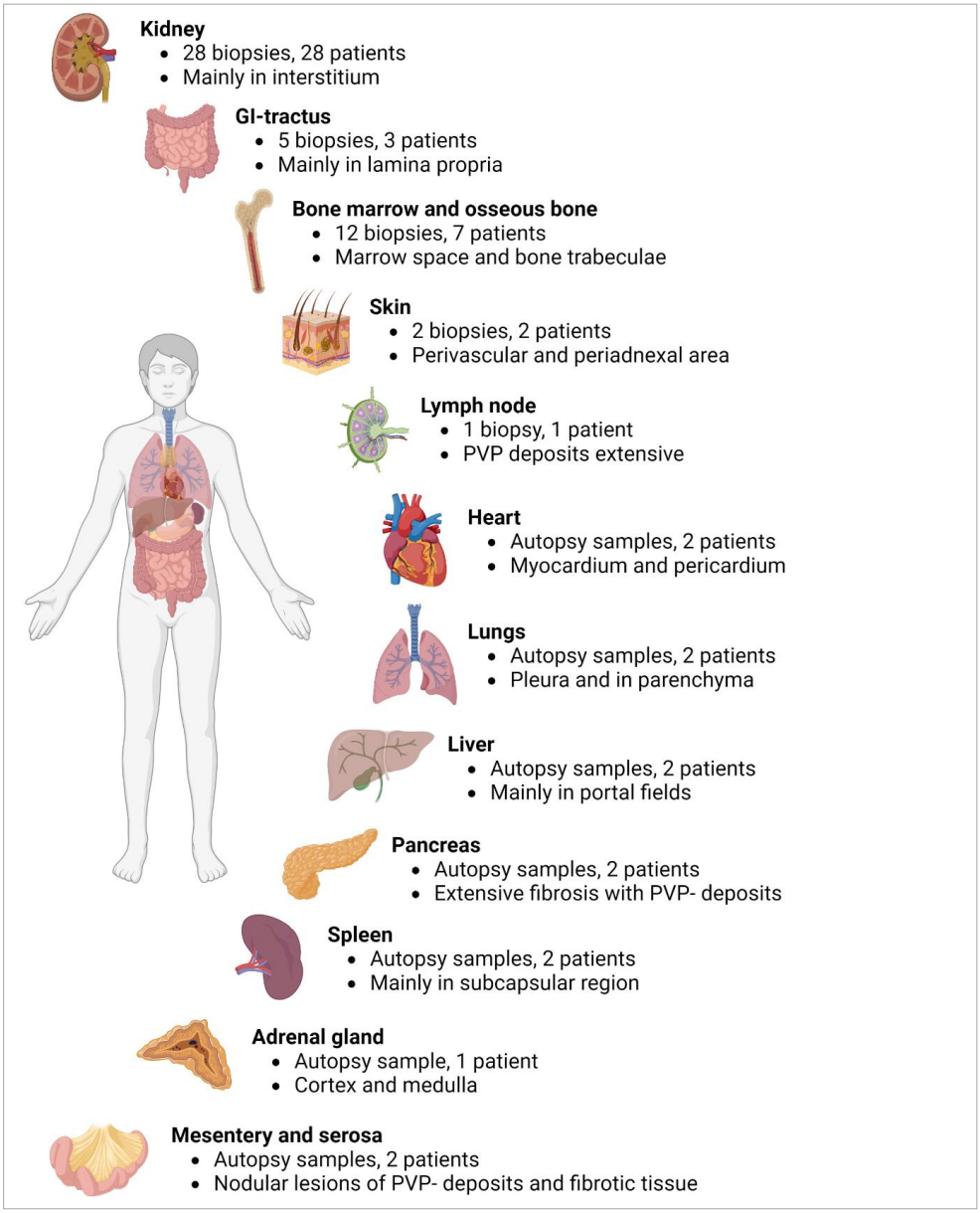

Figure 10: Overview of pathological findings in biopsies and autopsies *(number of instances, number of patients and localization or extent of deposits for each tissue). Deposits in kidney, GI-tractus, bone marrow and lymph nodes were also evident in one or both autopsies. Created with BioRender.com*

4.1 Paper 1 and paper 2

Paper 2 discusses the clinicopathological findings of 13 patients (31 biopsies and 2 autopsies) diagnosed with PVP deposition. The clinical course and findings of one of these patients are described and discussed in detail in Paper 1.

4.1.1 Pathological fractures and bone biopsies

Seven of the patients reported skeletal pain. Three patients suffered pathological fractures or bone destruction. One of these patients experienced multiple and ultimately debilitating fractures. Bone tissue biopsies were performed in all three cases. Pre-biopsy differential diagnoses were malignancy, osteomyelitis or necrosis of the femoral head based on radiological examinations. Biopsies (n=5) from the affected bone tissue revealed infiltrates of PVP-containing macrophages in the marrow space and in bone trabeculae. The marrow space showed extensive infiltrates of macrophages. Not all bone trabeculae were affected, but those that were showed reactive changes with loss of lamellar structure, ruffled edges and partial loss of osteocytes in lacunae, similar to findings in avascular necrosis. None of the biopsies showed evidence of malignant disease, osteomyelitis or any other disease that could explain the bone destruction.

4.1.2 Anemia and bone marrow biopsies

All 13 patients had some degree of anemia. Five of them received repeated erythrocyte transfusions and two had concomitant leukocytopenia or thrombocytopenia. Seven patients underwent bone marrow biopsy. Examples of pre-biopsy differential diagnoses were malignancy and histiocytosis. Bone marrow biopsies (n=7) showed infiltrates of PVP-containing macrophages in the marrow space. In four biopsies, the infiltrates almost completely replaced the hematopoietic tissue. All cell lines were present with a normal distribution in the scarce remaining hematopoietic tissue.

4.1.3 Abdominal complaints and biopsies from the GI-tractus

Nonspecific abdominal complaints, such as nausea, vomiting, constipation and diarrhea were common. Five patients also reported excessive weight loss of up to 20 kg in 6 months. Three patients underwent biopsy from the upper and/or lower GI-tract

(n=5). Indication for biopsy was anemia, weight loss or abdominal complaints. Biopsy findings included an extended lamina propria with infiltrates of PVP-containing macrophages with extension into the muscularis mucosa. Extension into the submucosa was also observed. Surface and crypt epithelium showed mainly normal findings.

4.1.4 Deaths and autopsy findings

At the time of the last follow-up in July 2020, six patients had died. Two patients presumably died from overdoses. Four other patients died with multiple organ failure. Among them, two underwent autopsy. Both autopsies revealed infiltrates of PVP-containing macrophages in all sampled organs (lungs, heart, liver, pancreas, spleen, kidneys, adrenal glands, mesenteric tissue, lymph nodes, and bone marrow). No other causative findings were made. We concluded, as did the autopsy reports, that PVP deposition had likely contributed to or caused the fatal outcome in these two patients.

4.1.5 The source of the deposited PVP

In 2013, at the time when the cases of PVP-deposition were recognized, three of the five OSDs marketed in Norway and several other oral drugs with addictive properties (e.g. oxycodone tablets) contained PVP as an excipient. As most of the patients in our material were receiving OST, we suspected the PVP-containing OSDs as potential sources. Two similar types of buprenorphine formulations and one methadone formulation contained PVP as an excipient. Information on the PVP content of the formulations is given in Table 3 in Section 1.4.

Based on the difference in molecular weight and the absolute amount of PVP in a typical dose, we favored the methadone syrup as the predominant source of PVP in these cases. To further support this assumption, we performed calculations based on our own morphological findings and previously published data (88, 102, 120).

Bone marrow biopsies from two of the patients showed almost complete occupation of the bone marrow space by PVP-containing macrophages. Making the rough assumption that the entire bone marrow mass of these patients was replaced by PVP, we were able to calculate their total body PVP load. We based this calculation

(Table 4) on the average bone marrow mass of male humans (3000 g) (119), the amount of PVP in PVP-saturated tissue (0.005 g /gram of tissue) (102), and the proportion of the total body PVP load deposited in the bone marrow (6.5%) (88).

Table 4: Calculation of total body PVP load in patients with severe PVP deposition in bone marrow biopsies.

Data	Calculation	Explanation
$0.005\ g\ PVP/g\ tissue$		PVP load in PVP saturated tissue
$3000\ g$		Average bone marrow mass in male humans
	$0.005\ g/g \times 3000g = 15\ g$	PVP mass in bone marrow
6.5 %		Proportion of bone marrow PVP to total body PVP load
	$(15\ g/6.5) \times 100 = 230\ g$	Estimated total body PVP load

With an estimate of the amount of PVP deposited in the most severely affected patients, we were able to assess whether it was reasonable that injection of the PVP-containing OSDs could cause this level of PVP accumulation. We assumed that 100% of the injected PVP K90 and 50 % (likely an overestimate) of the injected PVP K30 would be retained in the body (3, 88), and calculated the number of injections of each formulation required to cause an accumulation of 230 g of PVP (Table 5).

Table 5: Calculation of the number of doses injected to cause accumulation of 230 g of PVP.

Explanation	Buprenorphine tablet	Methadone syrup
Excipient	PVP K30	PVP K90
Mean MW	44 – 54 kDa	1 000 – 1 500 kDa
PVP content per standard dose	8 mg	585 mg
PVP assumed retained per dose	4 mg	585 mg
Number of injections needed for accumulation of 230 g PVP	57 500	392

The calculation showed that patients would have had to inject 392 doses of the methadone syrup or 57,500 doses of buprenorphine tablets to achieve a total PVP body

load of 230 g. Although these are estimates, the results leave little uncertainty that the methadone syrup containing PVP K90 is by far the most likely source of PVP in these cases.

4.2 Paper 3 – CKD and renal PVP deposition

Paper 3 describes and discusses in detail the clinicopathological findings in patients with kidney insufficiency and kidney PVP deposition.

4.2.1 Clinical findings at time of biopsy and patient outcome

28 kidney biopsies with PVP deposition were identified. All 28 patients had a history of injecting drug use and opioid addiction. Hepatitis B and C virus positivity was common, but no patient was HIV positive. All patients had CKD or newly discovered kidney insufficiency. Mean s-creatinine and eGFR levels at the time of biopsy were 2.45 mg/dl (217 µmol/L) and 33 ml/min per 1.73 m^2, respectively. Most patients either did not show proteinuria or had mild proteinuria. Nine patients had moderate proteinuria and one patient presented with severe proteinuria. No patient had nephrotic range proteinuria.

Follow-up data were available for 26 patients. For 23 patients (88.5 %), eGFR had slowly declined, stabilized or improved, but normalization of eGFR was not observed. Three patients had rapid decline in eGFR, of which two patients had developed end-stage kidney disease requiring hemodialysis.

4.2.2 Light microscopical findings

All kidney biopsies showed an extended interstitium with varying degrees of fibrosis and moderate or severe tubular atrophy (TA). The mean extent of tubular atrophy was 65 % (SD 19.9, range 34 – 92). Singular or clustered PVP-containing macrophages were scattered in the extended interstitium, and in some cases infiltrates of macrophages displaced the tubuli in larger areas. We could not find a significant correlation between the extent of TA and the extent of PVP deposition.

Only two biopsies showed evidence of glomerulonephritis, one of which was from a patient with previously diagnosed systemic lupus erythematosus. Mean share of

glomeruli with global sclerosis was 8% (SD 11, range 0 – 50). The most striking finding in the glomerular compartment was the large number of underperfused and ischemic glomeruli, corresponding to a low mean glomerular volume. In 22 cases, we observed PVP-containing vacuoles in glomeruli by light microscopy.

Arteriosclerosis and/or arteriolohyalinosis was evident in 27 of 28 cases, and moderate to severe vascular sclerosis was common. We found no significant correlation between the severity of vascular sclerosis and the extent of TA or the share of underperfused or ischemic glomeruli.

We did not find any of the common kidney diseases associated with IV drug use as described in 1.1.3 in this cohort of kidney biopsies.

4.2.3 Ultrastructural findings

EM was performed in 27 out of 28 cases. In 22 biopsies, we observed mesangial cells with vacuoles that met our criteria for PVP-containing vacuoles. Eleven biopsies also showed endothelial cells with PVP-containing vacuoles. In podocytes, vacuoles were typically fewer per cell, more variable in size, and collocated with osmiophilic material suspicious of lipofuscin, thus not fulfilling our criteria for PVP vacuoles.

5 Discussion

This project began with the discovery of the reappearance of a diagnosis thought to be of historical interest only. The origin of the project and the questions immediately raised by this discovery determined the aims of the studies that are the subject of this thesis. Where did the deposited PVP come from? How extensive are these deposits? Have they caused disease or even contributed to fatal outcomes?

One problem with studying a "historical diagnosis" is that the literature to which we compare our findings is also "historical". Most of the literature describing PVP deposition is more than 50 years old, and some of it is written in French or German. To the best of our knowledge, PVP K90 has not previously been used in parenteral medication, and it is uncertain whether this affects the comparability of our findings with those of previous literature.

Although the modern pathology textbook Enzinger & Weiss's Soft Tissue Tumors (1) includes a section on "polyvinylpyrrolidone granuloma", few pathologists would recognize PVP deposits as such. This gap in information about PVP deposition and the nature and severity of disease in some of these patients demonstrated the need for the studies and publications that form the basis of this thesis.

Our choice of methods, our results and the relevance of our studies are discussed in the following chapter.

5.1 Discussion of material and methods

5.1.1 Collection of cases

In 2013, when the first cases of PVP deposition in individuals with IVDU were recognized, most pathologists were unfamiliar with this disease entity. We identified biopsies received prior to 2013 that showed PVP deposition. Although the infiltrates of vacuolated macrophages were often a major finding in these biopsies, the origin of this finding was rarely addressed and the diagnosis of PVP deposition was not made at the time of primary evaluation. To ensure that PVP deposition was identified in the future, information about the findings was distributed to colleagues at the Department of

Pathology at Haukeland University Hospital at local meetings. Attention to this phenomenon was high at the time, and it is likely that most biopsies with PVP deposition received by the department after this were correctly diagnosed. However, we did not perform a systematic search for cases in our departments' archive, as this would have been almost insurmountable compared to the information it would have yielded. It is therefore likely that there are undiagnosed cases, also among the population served by Haukeland University Hospital.

Information about PVP-deposition was also shared with colleagues at other pathology departments in Norway, and ten kidney biopsies were sent to us either for second opinion evaluation or for the sake of the research project. Despite the efforts to disseminate information, the level of attention to PVP deposition was likely lower at other pathology departments. As we did not make a systematic call for cases, it is likely that there are both undiagnosed and diagnosed cases of PVP-deposition in other parts of Norway of which we are unaware.

The unsystematic approach to case collection makes our studies unsuited to describe differences in the occurrence of PVP deposition across time and different regions of Norway.

Kidney biopsies represent a high proportion of the biopsies in our research material. This could be taken as an indication that PVP deposition disproportionately affects the kidney. However, several structural factors could explain this overrepresentation. As the project leader, Sabine Leh, is a nephropathologist, it is likely that the reach of information in this specific field of pathology was greater both at the department at Haukeland University Hospital and in other regions. This may have resulted in a higher rate of detection of PVP deposition in kidney biopsies. In addition, the reach of information to the clinical nephrology community was likely greater than to other clinical disciplines. It is possible that attention to renal PVP deposition in persons with IVDU led nephrologists to perform kidney biopsies more frequently in this patient group, at least at Haukeland University Hospital. Combined, the above-mentioned factors suggest that a selection bias is likely one of the reasons for the over-representation of kidney biopsies in our material.

In Norway, the clinical and pathological findings of all consenting patients undergoing a non-neoplastic kidney biopsy are recorded in the Norwegian Renal Registry. Cross-linking the Norwegian Renal Registry with the Norwegian Patient Registry for patients enrolled in OST would be a more systematic approach for finding eligible kidney biopsies in the search for cases of PVP-deposition. However, obtaining permission to cross-link such registries and retrieving the data is time-consuming and we judged that it was not feasible in the time frame of the project.

Although our collection of cases is likely incomplete, it is relatively large considering the rarity of the disease, especially for kidney biopsy cases.

5.1.2 Tissue samples and clinical data

All tissue and clinical data evaluated in our studies were collected as part of the diagnostic or follow-up care of patients in the health-care setting.

The tissue samples available to us were archived diagnostic biopsies and autopsy specimens. The use of archival material is a gentle and accessible way of doing pathology research. It was not feasible or ethically justifiable to collect tissue samples from patients for the purpose of this project. However, the use of archival material comes with some limitations. Archived tissue is essentially the property of the patient and exhausting the remaining tissue for the purpose of research is not recommended. In addition, there were a small number of biopsies where tissue blocks were missing, so the existing tissue slides and analyses were the only ones we had at our disposal. These two issues limited the number of analyses that could be performed for each biopsy. However, we believe it is unlikely that they would have affected the results of the studies.

We gathered the clinical data from digital patient records, pathology referral forms and from the Norwegian Renal Registry. We only had access to the digital patient charts of patients belonging to the Western Norway Health Region. Specified clinical information about patients belonging to other health regions was provided upon request. This may imply a difference in the detail of clinical information for patients within and outside of the Western Norway Health Region and represents another potential source of bias in our studies.

Apart from the problem of collection bias, the retrospective nature of our studies leaves us without valuable information. Information such as the amount of methadone injected, the types of drugs consumed and the duration of IVDU would be valuable information in our interpretation and understanding of the findings. Obtaining this information from the patients would likely be very difficult, partly because of the unstable living conditions of many in this patient group. In addition, self-reporting on substance use may lead to under-reporting and information being withheld for fear of repercussions (128).

5.1.3 Identification of PVP in tissue samples

In our studies, we based the identification of PVP deposits solely on the morphological appearance in tissue slides stained with H&E, Congo red and PASM. The findings were consistent with the literature describing PVP deposition (102). However, it is possible to detect the presence of PVP in tissue samples and to determine the type of PVP present by mass spectrometry (84). The analysis is preferably performed on fresh frozen tissue, which was not available to us. Another problem was that the Norwegian facilities were reluctant to analyze our tissue samples. Eventually, one of the producers of PVP, BASF Ludwigshafen, offered to perform a gel permeation chromotography on a sample of synovial fluid. PVP K90 was detected, but it represented only 8.7 % of the PVP present. The main part of the PVP present had a narrowly distributed molar mass of 35 000 g/mol (personal communication Yvonne Matheis, Competence Center Analytics, BASF, 21.10.2015). The origin of this fraction of PVP in the synovial fluid sample could not be determined. Because of these conflicting results and the lack of fresh frozen tissue, we decided not to pursue the quantitative detection of PVP in tissue any further and continued to rely on the morphological findings for the detection of PVP.

5.1.4 Calculation of total body PVP load

In 2013, it was already suspected that the methadone syrup containing PVP K90 was the main source of PVP in the patients studied. As described above, it was challenging to perform a tissue analysis that could confirm the presence of PVP with MW in the range of PVP K90. Therefore, we performed calculations to estimate the total body

PVP load in some patients to assess the likelihood that other PVP-containing medications could cause the extent of PVP deposition observed. In order to perform these calculations, we had to make several assumptions. These assumptions have probably made the results less accurate. Based on bone marrow biopsy findings in two patients, we assumed that PVP deposits replaced the entire bone marrow mass in these two patients. Although these two patients had clinical signs of bone marrow failure, it is problematic to make this assumption based on the findings in one small biopsy. There may also be differences between the extent of deposition in red and yellow bone marrow. To calculate the amount of PVP retained in the bone marrow, we used data from Mechanik on the average bone marrow mass of adult males (120) and data from Reske-Nielsen et al. on the concentration of PVP in PVP-saturated tissue (102). Although the reliability of Mechanik's data has been questioned, his results are still used as a basis for comparison in modern studies on the distribution of bone marrow mass in humans. The results of Reske-Nielsen et al. were based on spectromorphometric analysis of a subcutaneous nodule with extensive PVP deposition (102). Even though we have no reason to question their results, it is questionable whether the results concerning deposition of moderate MW PVP in subcutaneous tissue can be extrapolated to the deposition of high MW PVP in bone marrow. With the estimate of the amount of PVP retained in the bone marrow, we were able to calculate the total body PVP load using previously published data on the distribution of retained PVP in different organs (88). Again, we had to assume that findings on the deposition of moderate MW PVP would be applicable to the deposition of high MW PVP. In addition, the study on the distribution of retained PVP suggested that there is a redistribution of retained PVP over time, a factor that we were unable to account for in our calculations.

Collectively, these assumptions make the calculations of the total body PVP load inaccurate and the results must be considered as estimates. However, we believe that the calculation of the number of injections of the marketed OSTs required to achieve this total body PVP load, is still highly relevant. Despite their inaccuracy, these calculations were reasonable proxies in the situation where we were unable to quantitatively verify the presence of PVP K90 in tissue samples.

5.1.5 Methods performed on kidney biopsies

Extent of PVP deposition

In order to quantify the extent of PVP deposition in kidney biopsies, we calculated the percentage of the total biopsy area occupied by PVP-containing vacuoles. The area of PVP-containing vacuoles was measured by manually encircling the PVP-containing vacuoles on digitalized tissue slides stained by Congo red or H&E when Congo red was not available or technically failed. Several factors might have biased the results: First, differences in the staining characteristics between Congo red and H&E may have had an impact on area measurements. In addition, as the measurements were performed by two different investigators, inter-observer variation as well as intra-observer might have influenced the results. Despite these potential biases, we do not think that the results were significantly affected as both stains clearly identified the vacuoles.

The variability introduced by manual annotation might have been avoided by using automatic image analysis on digital slides. However, there are some obstacles which suggest that this method would not be feasible. In the H&E stain, PVP deposits are identified by the faint blue-greyish stain combined with the typical texture of the deposits. Image analysis would utilize only the faint color of the deposits for identification, not the texture. We assume that the low contrast between the PVP deposits and the surrounding tissue would not be sufficient to tweak an image analysis algorithm to reliably detect the deposits. Utilizing Congo red stained tissue slides or a macrophage marker such as CD68, might have resolved this problem. However, in our experience, there was a considerable variation in the staining characteristics of PVP-containing vacuoles with Congo red stain, both in terms of hue, intensity and saturation. The variation in color would limit the applicability of automatic image analysis. It would have been technically easier to quantify the deposits by using the brown color produced by an immunohistochemical macrophage marker such as CD68. However, the disadvantage of this method is that quantification would include areas with increased macrophage numbers for other reasons, such as areas of tubular atrophy

or degeneration. Therefore, image analysis of CD68-stained tissue slides would overestimate the extent of PVP deposition.

In conclusion, despite being time-consuming and encumbered by intra- and inter-observer variability, manual detection was considered the most appropriate method for quantifying the extent of PVP deposition.

Quantification of tubular atrophy

It has long been recognized that the extent of tubular atrophy and interstitial fibrosis correlates very well with clinical parameters of kidney function such as serum creatinine or GFR (129, 130). Tubular atrophy and interstitial fibrosis are key parameters in the diagnostic evaluation of kidney biopsies, as they are also strong prognostic factors (131, 132). Tubular atrophy and interstitial fibrosis usually occur together and are highly correlated (133, 134). In the diagnostic setting, the extent of tubular atrophy and interstitial fibrosis is commonly evaluated by visual assessment of PAS- and trichrome stained tissue slides, although methods differ between pathology departments. With visual assessment, the pathologist roughly estimates the extent of tubular atrophy and interstitial fibrosis as mild (<25 %), moderate (25-50 %) or severe (>50 %) simply by looking at the cortical tissue (135). Another method used is to estimate these parameters in 10% steps in consecutive high power fields throughout the cortex and then calculate a mean of the estimates. Visual assessment is an effective way to evaluate the extent of tubular atrophy and interstitial fibrosis. However, drawbacks include the grouping into categories and the high inter-observer variability found in some studies (136).

Tubular atrophy was a central finding in our kidney biopsies, and we suspected that the extent of tubular atrophy would correlate with the extent of PVP deposition. Therefore, we wanted to find a more accurate estimate of the extent of tubular atrophy than visual assessment could provide. The point-counting method, described in section 3.5.1, is an unbiased morphometric method for measuring the extent of pathological findings in tissue slides and is commonly used in scientific settings (122). We measured the extent of tubular atrophy by calculating the fraction of points hitting areas with tubular atrophy out of all points hitting the entire cortical tubulointerstitial

area, excluding glomeruli and large vascular structures. This area includes not only atrophic tubules, but also the interstitium, which was usually extended. Alternative measurements could have been applied to estimate the extent of tubular atrophy, such as counting the fraction of points hitting areas with tubular atrophy out of all points hitting the entire cortex including glomeruli and vascular structures (137). As the area fraction of structures other than the tubulointerstitial area might vary from biopsy to biopsy, we considered that excluding this area was the most reliable approach to estimating of the extent of tubular atrophy.

Even though the area measured includes the interstitium, our method does not measure the extent of interstitial fibrosis, which often, but not always accompanies tubular atrophy. This is because the composition of the interstitium may vary. In addition to fibrosis, edema and inflammation may also contribute to expansion of the interstitium as well as the PVP deposits. We assessed interstitial fibrosis only semiquantitatively on trichrome-stained slides believing that a quantitative assessment of tubular atrophy provided sufficient information on chronic kidney damage.

Glomerular volume estimation

We estimated individual glomerular volumes using the Weibel-Gomez method (124). This method estimates the volume of a structure from its cross-sectional area in a single tissue section. For glomeruli, it assumes a spherical shape and a certain size distribution. As the shape and size distribution of glomeruli is not uniform, these assumptions make the estimated volume inaccurate. The Weibel-Gomez method tends to overestimate the glomerular volume compared to the Cavalieri method, which is considered the gold-standard method for estimating glomerular volume (125). The Cavalieri method avoids the problem of assumptions of shape by measuring the glomerular profile area in multiple consecutive sections of standardized thickness (125). The method is very time- and resource-consuming. In our opinion, the reward of a more accurate estimate of glomerular volume would not be proportionate to the resources required to perform it. In addition, the amount of tissue in a diagnostic kidney biopsy is limited and exhausting the tissue for this purpose would be inappropriate.

EM methods

To distinguish PVP-containing vacuoles in glomeruli from vacuoles containing other materials, we developed a set of criteria (see 3.6). These were based on our own observations, descriptions of the ultrastructural appearance of PVP deposits in the literature (105, 127), and comparisons between the ultrastructural appearance of vacuoles in recognized cases of PVP deposition and vacuoles in kidney biopsies from patients without PVP exposure. We identified vacuoles meeting these criteria in mesangial cells and endothelial cells, but not in podocytes. However, our criteria have not been validated for their ability to discriminate PVP-containing vacuoles from vacuoles not containing PVP. Nevertheless, our findings are in line with the understanding that the deposited PVP are of a high MW and thus too large for glomerular filtration.

5.2 Discussion of main findings

In **Papers I-III**, we reported the clinicopathological findings in 33 patients with PVP deposition. Our findings and their possible implications are discussed below.

5.2.1 The source of PVP

Prior to 2014, both buprenorphine tablets and a methadone syrup containing PVP were marketed in Norway. Several of the patients admitted to injecting both the methadone syrup and the buprenorphine tablets. In all three publications, we stated that the methadone syrup containing PVP K90 was the most likely source of the deposited PVP in the reported patients. This assumption was mainly based on the differences in molecular weight and absolute amount of PVP in the potential sources. The methadone syrup in question was withdrawn from the market in Norway and the EU in 2014 based on these facts (116).

In **Paper II,** we described the calculations we performed to support this assumption. The calculations showed that it would take the injection of well over 50 000 doses of buprenorphine, as opposed to approximately 400 doses of the methadone syrup containing PVP K90, to cause the level of accumulation observed in some patients.

The calculations were based on our own observations in biopsies and data from previously published studies (88, 102, 119). As described in the discussion of methods, we had to make several assumptions. Therefore, the results should be considered as estimates. However, the enormous difference in the number of injected doses required leaves little doubt that the methadone syrup containing PVP K90 was the predominant source of PVP for our patients **(Aim 1)**.

However, we cannot exclude, the possibility that injection of buprenorphine tablets or other medications containing PVP K30 may cause some accumulation and deposition. PVP K30 has a main molecular weight range (44 – 54 kDa) that allows for renal filtration, but filtration occurs over a period of time. In addition, PVP K30 products may contain a fraction of high MW PVP molecules (84) that cannot be excreted due to their MW. A study on the renal excretion of PVP showed that PVP was still being excreted in the urine 96 hours after a single intravenous infusion of C^{14}-labeled PVP K33. (88). One of the human subjects died from unrelated disease two months after administration of 17.5 grams of PVP K33, and the tissues were examined for C^{14} radioactivity. They detected C^{14} radioactivity in tissue samples from most organ systems and concluded that PVP deposition was present. The authors did not mention any clinical side effects from the infusion or evidence of organ damage from PVP deposition. There are examples in the literature of clinically relevant PVP deposition following injection of extended-release medication containing PVP with a MW equivalent of PVP K30 (2, 102-104). These are mostly cases of PVP deposition following frequent (e.g. daily) injections of PVP-containing parenteral medications over several years with a total administered dose of up to 3 kg (107). These formulations for injection are no longer marketed.

As PVP K30 is an excipient in several oral medications with addictive properties, such as buprenorphine, an important question is whether injection of such medications could cause PVP deposition. In the design and production of oral tablets, PVP K30 is often utilized as a binder in the wet-granulation process and the concentration of PVP is typically 2-5 % (84). Therefore, the absolute amount of PVP administered by a tablet would be only a few milligrams. However, frequent injections of oral

medications are not uncommon among persons with IVDU (35). This fact, combined with the slow excretion of moderate MW PVP and a high MW fraction in PVP K30, makes it at least theoretically possible that injections of such tablets could cause PVP deposition detectable in biopsies. However, we consider it very unlikely that it could cause the extent of PVP deposition seen in our cases.

5.2.2 Clinical consequences of PVP-deposition

The fact that moderate and high MW PVP is partially or completely retained in the body after parenteral administration has been known since the 1950's. The initial studies demonstrating PVP-deposition following intravenous injection of PVP found no or only minor or transient adverse effects from the deposition (2). Hence, whether PVP-deposition can cause clinically significant organ damage, is controversial. In **Papers I - III**, one of our aims was to explore whether the PVP deposition had caused organ dysfunction and disease.

Paper I is a case report describing the clinical course and pathological findings of one of the most severely affected patients. Although case reports rest at the bottom of the pyramid of evidence in medical research they can fulfil important functions. Cases can be "the first line of evidence … where everything begins" (138). They play a role in the recognition of rare disease manifestations and in the detection of adverse drug reactions. Our case illustrates this very well: The cause and clinical significance of the histological changes due to PVP deposition were recognized through a series of individual cases. These cases allowed us to discover an unexpected side effect of a drug being used inappropriately in the specific context of drug addiction. Case reports also allow a more detailed view on particularly complex clinical settings, which was the case for the patient in question. He suffered from a number of conditions, some of them debilitating, which were likely associated with PVP deposition. Although the study of his clinical course and pathological findings cannot be considered as evidence, it gives insight into the extent of PVP deposition and the suffering that PVP deposition likely caused **(Aim 2)**.

In **Paper II**, we studied the 30 biopsies, two autopsies and clinical data from 13 patients. Anemia, pathological fractures, abdominal complaints and kidney insufficiency were the most common indications for biopsy.

Anemia and bone marrow biopsy findings

All 13 patients had some degree of anemia. Seven patients underwent bone marrow biopsy because of anemia and, in some cases, pancytopenia. All biopsies showed PVP-deposition in the bone marrow space, with an extent varying from sparse to almost complete replacement of the hematopoietic tissue. Similar findings have previously been reported in four cases (106, 113, 114). All cases showed anemia and one case showed pancytopenia, and bone marrow biopsies showed almost complete replacement of the hematopoietic tissue by PVP-containing macrophages. Another reported case showed less extensive PVP deposition in the bone marrow biopsy, and accordingly the patient did not have anemia or cytopenia. (102). The pathophysiological mechanism by which PVP deposition may cause bone marrow dysfunction is unknown. Morphologically, there is little inflammatory response to the deposits. Based on our own findings and the few previously reported cases, there appears to be a relation between the extent of bone marrow deposition and the severity of anemia and cytopenia. One possible mechanism is that PVP deposits cause damage simply by occupying the marrow space and thereby replacing the hematopoietic tissue. On the other hand, it should be noted that anemia is common in the population of persons with IVDU in part due to frequent injections and chronic infections (139). Therefore, the observation of anemia in all 13 patients in paper II is not surprising and could be explained by this. However, the severe anemia observed in four patients and the pancytopenia in two of them cannot be explained by these factors. At least in these four cases, it is highly likely that the extensive PVP deposition in the bone marrow had caused the anemia and cytopenia observed **(Aim 3)**.

Pathological fractures and osseous bone biopsy findings

Three patients suffered one or more pathological fractures or bone destruction, and a total of five biopsies were taken from the affected bone. All biopsies showed PVP-containing macrophages in the marrow space and partially in the bone trabeculae with

reactive changes similar to those seen in avascular necrosis. Pathological fractures associated with PVP-deposition in trabecular bone have previously been described in three cases (102, 112, 115). Two of these patients had received intravenous injections of PVP-containing solutions for several years for nutritional support and for blood pressure correction (112, 115). The third patient had received daily subcutaneous injections of a PVP-containing vasopressin analog for 25 years (102). None of the publications reported the MW of the injected PVP. All patients suffered one or more pathological fractures, and biopsies from the affected bone showed findings similar to ours. One of the publications postulated that PVP deposition had caused mucoid softening of the bone trabeculae (112). Another possible mechanism could be that the extensive infiltrates of macrophages caused hypoperfusion of osseous tissue leading to avascular necrosis. This is one of the proposed mechanisms of avascular osteonecrosis seen in Gaucher's disease, another macrophage storage disease affecting the bone marrow (140). Although the pathological mechanisms are unknown, we concluded that it is highly likely that PVP deposition contributed to the bone destruction and fractures in our patients (**Aim 3**).

Abdominal complaints and GI-tract biopsy findings

Nonspecific abdominal complaints were common among the patients reported and five patients had experienced severe weight loss of up to 20 kg in six months. A total of five biopsies from the upper and lower GI-tract were taken from three of the patients. These biopsies showed infiltrates of PVP-containing macrophages in the lamina propria with extension into the muscularis mucosa. Two case reports have previously described PVP deposition in the gastrointestinal tract (103, 141). The case report by Hewan-Lowe et al. described PVP deposition in the gastric mucosa of a patient with abdominal pain and radiological evidence of a poorly distensible antrum (141). In this case, the PVP deposits in the gastric mucosa were misinterpreted as poorly differentiated mucin-producing signet ring cell carcinoma, leading to an unwarranted total gastrectomy. Fortunately, there have been no misdiagnoses of malignancy in the cases we reported. However, the infiltrates of PVP-containing macrophages, although often the main finding, were not recognized as such in biopsies evaluated prior to

2013. The case report by Edelmann et al. describes a patient who had been treated with daily injections of PVP-containing formulation for 15 years (103). He experienced abdominal pain and vomiting, and exploratory laparotomy revealed upper gastrointestinal tract obstruction due to massive PVP-deposition in the mesentery and retroperitoneum. We did not observe incidents of gastrointestinal tract obstruction in the patients reported in our studies. However, we did observe peritoneal nodules with PVP deposits in the peritoneum in both autopsies.

Opioids have well-known gastrointestinal side effects, including constipation, nausea and vomiting, collectively known as opioid-induced bowel dysfunction (142). Unlike the analgesic effects of opioids, opioid-induced bowel dysfunction does not lead to tolerance and is prevalent in persons with opioid addiction. In an observational study of 1057 persons receiving OST, the authors reported a 53 % prevalence of moderate to very severe constipation (143). Hence, the complaints of abdominal pain and vomiting among the patients reported in our studies could be partly or completely related to opioid side effects. However, some of our observations suggest that PVP deposition may have contributed to the abdominal symptoms **(Aim 3)**. The excessive weight loss observed in five patients is difficult to explain by opioid-induced bowel dysfunction alone. PVP deposition may impair absorption, as seen in patients with gastrointestinal involvement of Langerhans histiocytosis (144). Involvement of the pancreas, as seen in both autopsies, may also contribute to weight loss. Finally, we cannot exclude that the nodular lesions in the peritoneum seen in both autopsies might have contributed to the abdominal discomfort.

CKD and kidney biopsy findings

In **Paper III,** we studied the clinical findings and pathological findings in kidney biopsies of 28 patients with CKD and PVP deposition.

All patients had laboratory parameters indicating kidney insufficiency (mean s-creatinine 217 µmol/L, mean eGFR 33 mL/min/1.73 m^2). All biopsies showed interstitial infiltrates of PVP-containing macrophages, mainly in areas with tubular atrophy. EM also showed PVP-containing vacuoles in the glomerular mesangium and/or glomerular endothelial cells in 22 of 27 cases, but not in podocytes. In addition

to PVP deposition, the main biopsy findings were moderate to severe tubular atrophy (mean area 65 %), collapsed glomeruli due to ischemia, and atherosclerosis and arteriolosclerosis.

Renal PVP deposition following parenteral administration of moderate MW PVP has previously been described in several reports (98, 105, 106, 145). In contrast to our cases, the most commonly reported finding was vacuolar swelling of the tubular epithelium (98, 145), a finding that can likely be ascribed to osmotic nephrosis. Osmotic nephrosis is a morphological pattern showing vacuolar swelling of the tubular epithelial cells (146). The vacuoles originate from pinocytosis of exogenous material (e.g. mannitol) from the tubular lumen. For this to occur, the exogenous material must first be filtrated by the glomerulus, which is possible for moderate MW PVP but not for PVP K90. Therefore, the discrepancies between the kidney findings described by us and those described in previously published cases can be explained by the difference in the MW of the PVP administered. However, a case report published by Bert et al. describes findings very similar to ours in a kidney biopsy from a patient with kidney insufficiency (106). The patient had received an estimated total amount of 3 kg of moderate MW PVP through daily injections of a PVP-containing formulation. The kidney biopsy showed both interstitial and glomerular PVP deposits, albeit also present in podocytes (106). Although the amount and MW of PVP injected differed from our cases, this case report shows that similar kidney changes in association with PVP have previously been observed and that they were related to kidney insufficiency.

Like the above-mentioned studies, the nature of this study, being a retrospective observational study, makes it ill-equipped to prove causal relationships. Our perception was that the PVP deposition had caused the tubular atrophy and kidney insufficiency. We expected to find a correlation between the extent of PVP deposition and the extent of tubular atrophy to support this perception, but we did not.

The lack of a significant correlation might have several explanations. First, the study may have been underpowered. Secondly, there may have been a redistribution of PVP from the renal interstitium to other sites in the body, as suggested in previous studies (88, 147). Nevertheless, a significant correlation would not have proved a causal

relationship just like the lack of one does not prove there is none. Hence, it cannot be known with certainty whether the PVP deposits played a role in the development of kidney insufficiency in our patients. However, our view is that the uniform kidney biopsy findings strongly support PVP as a main factor in the development of atrophy and kidney insufficiency.

Another interesting question is how PVP deposition might cause tubular atrophy. One possible pathophysiological mechanism could be that the spatial effect of interstitial PVP deposits contributes to peritubular rarefaction, thereby impairing the supply of nutrients and oxygen to the tubules. Another mechanism may be that the PVP-containing macrophages induce a pro-inflammatory response inducing tubular injury. Studies in recent decades have demonstrated the importance of macrophages in tubular injury and repair and that, depending on macrophage activation, they can contribute to both healing and further injury (148). Although our study cannot confirm any of these possible mechanisms, the frequent finding of underperfused glomeruli is consistent with the assumption that tubular atrophy caused reduced glomerular filtration through tubuloglomerular feedback and was thus central to the development of kidney insufficiency in our patients.

Although we consider it likely that the PVP-deposition contributed to kidney insufficiency in our patients, persons with IVDU are at risk of kidney disease through several mechanisms, as mentioned in the introduction (Section 1.1.3.). 96% of our patients were seropositive for HCV, and HCV-associated glomerulopathy was often a differential diagnosis suggested by the clinician. The question of amyloidosis was another frequent enquiry. However, none of the biopsies showed either of these findings. Other findings commonly seen in this group of patients, such as acute tubular necrosis or interstitial nephritis (except for the infiltrates of macrophages), were not seen. Arteriosclerosis was a common finding in our cases and could potentially provide an alternative explanation for the widespread tubular atrophy. Arteriosclerosis seems to be common in persons with IVDU as shown in the autopsy study by Buettner et al, who reported a high prevalence of at least moderate arteriosclerosis and showed an association with cocaine use. (56). However, when comparing the extent of tubular

atrophy in the autopsy study with our findings, it becomes apparent that the tubular atrophy in our study is disproportionately more extensive compared to the severity of the arteriosclerosis. This discrepancy supports that vascular alterations alone are insufficient to account for the extent of tubular atrophy in our biopsies.

Taken together, the lack of other explanatory findings in our kidney biopsies strengthens our view that PVP deposition substantially contributed to the development of kidney insufficiency in our patients **(Aim 3)**.

5.3 The relevance and value of our studies

As the source of PVP to our patients, the methadone syrup containing PVP K90, was withdrawn from the market in Norway and Europe in 2014, one might argue that PVP deposition is once again a historic diagnosis. Since then, as expected, new cases of PVP deposition have been rare, although they have not disappeared completely. Of note, PVP deposition is still seen in new biopsies from patients previously diagnosed with PVP deposition. As mentioned in section 5.2.2, oral medications with addictive properties, including OSDs, containing PVP K30 are still on the market. Although it is very unlikely that injection of such medications could cause the level of deposition seen in our patients, it could potentially cause deposits visible in biopsies. Detection of such deposits might be a clue in revealing IVDU.

While the cause of this new wave of PVP deposition disease has been eliminated, our findings may have relevance in other contexts. Despite the fact that the observation of foreign material in tissue samples from patients with IVDU is quite common, it is often not investigated further, and its potential significance therefore not emphasized. This project is an example of how dwelling on such findings can sometimes make a difference. Furthermore, the project highlights the important role pathologists can play in identifying and reporting potential adverse effects.

Our findings can also serve as a cautionary tale for the pharmaceutical industry. Obviously, medications designed for oral administration are formulated in a way that is appropriate for this mode of administration. However, caution should be exercised

when designing medications with addictive properties, particularly OSDs, which are prescribed to a population with a high prevalence of IVDU. Although efforts to prevent injection of OSDs are justified, these efforts should be made with the awareness that they may fail. We do not know what steps were taken to ensure that the safety of the methadone syrup was maintained when PVP K90 was added. However, we can speculate that user representatives and other stakeholders were not involved in a manner that would have allowed them to caution against the risk of injection despite the syrups' high viscosity.

As a group, persons with opioid addiction and IVDU have a high burden of somatic comorbidities, both drug-related and non-drug-related (149, 150). In part due to the success of OST, the population of persons with opioid addiction in Norway and other countries is ageing, and an increase in the prevalence of age-related chronic diseases can be expected. In addition, studies suggest that age-related chronic diseases occur earlier in drug-users than in the general population (151). Our studies have shown that drug-use, and especially IVDU can contribute to disease in most organ systems through unexpected mechanisms. In the follow-up of somatic complaints and disorders in patients with a history of drug use, differential diagnoses both related and unrelated to drug use should be considered. The pathologist should also bear this in mind when evaluating tissue samples from this patient group.

The pathologist is rarely a central actor in the follow-up of patients with drug dependence. However, this pathology-centered project has contributed to the identification and understanding of the cause of PVP deposition disease in patients with opioid addiction and IVDU. The discovery that prompted the initiation of this project led to the withdrawal of the methadone syrup that was the source of PVP. The withdrawal of this medication has likely prevented disease and deaths from PVP deposition among persons with opioid addiction and IVDU.

6 Conclusion

This thesis has summarized and discussed the findings in patients with opioid addiction, IVDU and PVP deposition. Our findings show very extensive PVP deposition in some patients, which likely contributed to the severe disease and fatal outcome in two patients (Papers I and II). Our estimates of total body PVP load indicate that the retained PVP most likely originated from injection of a methadone syrup containing PVP K90 (Paper II). Biopsies from the kidney, GI-tract, osseous bone and bone marrow were the most frequent in our material, but PVP deposition was found in most parenchymal and connective tissues (Paper II and III). Clinicopathological correlations strongly indicate that PVP deposition caused severe anemia and pathological fractures (Paper II). Although we could not find a statistically significant correlation to support this, the findings also suggest that renal PVP deposition has contributed to the development of CKD (Paper III).

In addition, this thesis highlights several points. First, it highlights the importance and value of a thorough pathology investigation. The occurrence of PVP-deposition in patients with opioid addiction and IVDU was discovered through attention to morphological findings in the diagnostic evaluation of tissue samples. Pursuing these findings led to the realization that an OSD was the most likely source of PVP. Second, the reporting of potential adverse drug reactions is valuable and can be relevant to the pathologist. The adverse drug reaction reporting systems in Norway and Europe are, in our experience, efficient and effective. The withdrawal of the methadone syrup containing PVP K90 in 2014 has probably prevented morbidity and deaths among persons with opioid addiction and IVDU. Finally, the PVP deposition disease in our patients might have been prevented if the methadone syrup had been designed with an awareness of the injecting behavior of its recipients and the potential harm that injecting PVP K90 could cause.

7 Future perspectives

Our findings show the extent of PVP deposition in individual patients. However, they do not allow conclusions to be drawn about the prevalence of PVP deposition in the population at risk. To elucidate this question, we plan to study the occurrence of PVP deposition in an autopsy material from persons with drug use autopsied at Haukeland University Hospital and the Forensic Department at the University of Bergen over a period of 12 years. REK has approved the study and data collection has begun. The archives of medical and forensic autopsies at these two institutions are searchable and complete archives of tissue samples from all autopsies performed to date exist. Searching the archives for eligible cases is feasible, as drug use is most often considered at least a contributing cause of death.

The scientific literature on kidney disease among persons with drug use is in part comprehensive, e.g. literature on HCV-related kidney disease. However, in our search for relevant literature, we found that there were few studies describing the range of kidney biopsy findings in this patient group. We believe that a descriptive retrospective observational study of the histological patterns and prevalence of kidney disease in patients with opioid addiction would be valuable, both scientifically and in the diagnostic setting. Finding relevant cases could be achieved by linking the Norwegian Patient Registry, the Norwegian Prescription Database and the Norwegian Renal Registry.

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9 Appendix

9.1 The story behind the project

The starting point of this project can be defined exactly: It was Thursday, January 3, 2013, in the shared office of the pathologists and spouses Sabine and Friedemann Leh at the Department of Pathology, Haukeland University Hospital. One of the advantages of a shared office is the ease with which you can consult each other. “Have you seen this before?” was Friedemann’s question to Sabine. He had been working with a bone marrow biopsy from the hospital in Førde. The bone marrow looked quite strange; it was stuffed with cells with big bubbles in the cytoplasm. Friedemann had never seen anything like it before. Sabine quickly looked at Friedemann’s biopsy in her microscope and asked: “Is the patient a drug addict?” Drug addiction was not mentioned in the requisition form. Friedemann called the responsible clinician in Førde, and in fact: the patient had been injecting drugs for a long time and was now in an opioid oubstitution oherapy (OST) program. Sabine is a nephropathologist and the background for her question was that she and her colleagues at the department had seen similar changes in some kidney biopsies. They could not explain the changes either, but all kidney biopsies had a common trait: They were from persons who had been injecting drugs.

Sometimes coincidence plays a big role, as it did here: Cecilie Askeland, at that time a resident doctor, came into the office because she had a question for Friedemann. Friedemann showed her the exciting case under the microscope. Cecilie saw the bone marrow with the bubbly cells and said: “We saw something similar in an autopsy two years ago. Those bubbly cells were everywhere in the body. We could not make a definite diagnosis”. They could not make a definite diagnosis, but they did a comprehensive evaluation of possible differential diagnoses including rare diseases such as Langerhans cell histiocytosis, mucopolysaccharidosis, Whipple’s disease, Erdheim Chester disease, and polyvinylpyrrolidone deposition disease. It was here, among these differential diagnoses, that Sabine and Friedemann became aware of the term “polyvinylpyrrolidone” (PVP, povidone). As PVP-related disease was considered

a consequence of infusion of plasma expanders that was merely of historical interest, the possibility of PVP deposition was not pursued further in the autopsy case. It was not surprising that this deceased person had also been injecting drugs and had received OST too.

Figure 11: "Revealed deaths". *Newspaper clipping from Bergensavisen (BA) 24.03.2014 showing Sabine and Friedemann Leh in their shared office at the Department of Pathology at Haukeland University Hospital. Photo by Linda Hilland. With permission from BA.*

Pathology can be as exciting as a detective story. The strange bubbly cells looked like the pictures of PVP deposition still described in modern pathology textbooks. There had to be a link between injecting drugs, OST and PVP deposition! Friedemann asked the right question: "Do OST drugs contain PVP?" It soon became clear to the pathologists, that PVP was widely used in the pharmaceutical industry: not as a plasma expander, but as an excipient in many medications. Opioid substitution drugs (OSDs) were no exception; some of them did contain PVP. PVP is not absorbed from the GI tract, but it is a rather common that OSDs are injected despite being designed for oral use. The final question was whether PVP could leave the body once it had entered the circulation. And indeed, only low and, to some extent, moderate molecular weight PVP could be filtered by the glomerulus, while high molecular weight PVP could not.

All this information gave the pathologists an explanation for why drug addicted patients might develop PVP deposits.

Still in January 2013, Friedemann made a call to the pharmacology department to ask for advice on what to do. He was referred to Tormod Bjånes at the "regionalt legemiddel informasjonssenter", RELIS. This was a difficult question for RELIS as the findings were certainly not a usual adverse drug effect, but rather the effect of an excipient caused by an unintended use of a drug. Finally, RELIS suggested using their form for adverse drug effects to report the findings as an effect of PVP. Sabine submitted the first reports on 28.08.2013 and RELIS received a total of 16 reports until April 2014.

Figure 12: "Concerned after several deaths". *Newspaper clipping from BA 21.03.2014 showing Hugo Torjussen, leader at "Strax-huset" in Bergen, a service for persons with drug dependence. Photo: Arne Ristesund. With permission from BA.*

Together with Tormod, Friedemann and Sabine established a working group to investigate the findings further. The working group contacted the Department of

Addiction Medicine at Haukeland University Hospital and asked for help. This brought Christian Ohldieck, then head of the department of OST, into the group. Another two clinicians joined the group: the nephrologist Einar Svarstad and the physician Gro Riise, who was treating one of the most severely affected patients. The group succeeded to narrow down the selection of PVP containing OSDs to the only one containing a high amount of high molecular weight PVP that could not be filtered in the glomerulus at all: Methadone Martindale syrup containing PVP K90 as an excipient.

From then on, the group worked along two lines: first to find a way to confirm that the "bubbles" in the biopsies in fact contained high-molecular PVP. And second, to sort out what to do with the findings.

To cut a long story short, the group never managed to analyze tissue specimens for PVP content but they could eventually detect PVP in the joint effusion of a patient. The following is a brief description of the attempts made: The usual response from Norwegian facilities to the request to analyze tissue samples by mass spectrometry was that they could not prioritize such an investigation. The PVP working group established a collaboration with a German research group who run MALDI mass spectrometry imaging on representative tissue sections. The results were inconclusive and confusing, as the analysis detected PVP both in PVP containing sections and negative control sections. The group also contacted BASF Ludwigshafen, the producer of PVP, and asked for help there. BASF generously offered assistance and suggested to run a Gel Permeation Chromatography analysis. Finally, in 2015, although not in tissue sections, PVP was successfully detected by gel permeation chromatography in the patient's synovial fluid. This fluid contained 0.83% PVP. The percentage of PVP K90 (the high molecular PVP in question, not filtered by the glomerulus because of the molecular weight) was 8.7%. The remaining PVP was a narrowly distributed PVP with a molar mass of about 35,000 g/mol, which could not be ascribed to PVP K30 due to the distribution. As is often the case, the result raised more questions than it solved. PVP K90 was detected but accounted for only a small proportion of the total

PVP detected. Where the vast majority of PVP with the narrow distribution spectrum came from remained unresolved.

Regarding the second aim – what to do with the findings, the group members found the link between OST, PVP depositions and disease and dead so strong, that they felt it right to contact relevant institutions and persons and report the findings. A series of meetings and presentations followed: first locally at the Department of Addiction Medicine, then at the national OST management meeting in Oslo. The pathologists also met the sales representative from Azanta, the company distributing Methadone Martindale in Norway, and informed him about the findings and a possible link to the Methadone syrup containing high molecular weight PVP.


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Forsiden nyheter

Det var på Haukeland sykehus patologer gjorde de alvorlige funnene. Foto: MAGNE TURØY

Trekker legemiddel fra markedet

Sannsynligvis er tre personer døde og 13 pasienter alvorlig syke som følge av å injisere stoffet Metadon Martindale, mener Statens Legemiddelverk.

Geir Jetmundsen Profil E-post

Linda Anette Hilland Profil E-post

Anbefal 149 0 +1 0 Skriv ut Tips en venn

Publisert 20.03.2014 kl 20:23 Oppdatert 21.03.2014 kl 09:37

Figure 13: "Withdraws drug from the market". *Newspaper clipping from BA 20.03.2014. The picture shows Haukeland University Hospital. Photo: Magne Turøy. With permission from BA.*

A turning point was the meeting with representatives from the Norwegian Directorate of Health and the Norwegian Medicines Agency 12.03.2014 in Bergen. The PVP working group informed the representatives of all the evidence they had uncovered so far. This meeting had several important consequences. First, the Norwegian Directorate of Health and the Norwegian Medicines Agency issued strong warnings against injecting Methadone Martindale on their websites 21.03.2014. Second, they announced that Methadone Martindale would be retracted from the Norwegian market 08.04.2014. Third, on 02.04.2014, the Norwegian Medicines Agency initiated an urgent union procedure and asked the European Medicines Agency (EMA) to review the risk benefit balance of all oral methadone medicines containing PVP in the European Union. The Pharmacovigilance Risk Assessment Committee (PRAC) then started a safety review. This included an ad hoc expert group meeting taking place in London at 14.06.2014 . Finally, EMA concluded its review in July 2014 and recommended that the oral methadone solutions containing high molecular weight PVP K90 should be suspended from the EU market.

I

CASE REPORT

REVISED Case Report: Polyvinylpyrrolidone deposition disease from repeated injection of opioid substitution drugs: report of a case with a fatal outcome [version 2; peer review: 2 approved]

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Abstract

Background: Intravenous injection of oral opioid substitution drugs (OSD) is widespread among injecting drug users. Several OSDs contain the polymer polyvinylpyrrolidone (PVP) as an excipient. Parenterally administered PVP of high molecular weight may accumulate in tissues and organs. This phenomenon was first described in the 1950s, when PVP was utilised in medication for parenteral use. We report a case of an opioid-addicted patient with extensive PVP–deposition caused by repeated injections of OSDs.

Case presentation: A 30-year-old male drug addicted patient in opioid substitution therapy (OST) was repeatedly referred to his local hospital in a poor general condition. Work-up revealed severe normocytic anaemia, renal insufficiency, pancreas insufficiency and pathological fractures. Biopsies from fractured bones, bone marrow and gastric mucosa showed extensive infiltrates of histiocytes with intracytoplasmic vacuoles. Vacuole content stained slightly bluish in hematoxylin and eosin stain, red in Congo red stain and black in periodic acid methenamine silver stain. The morphological appearance and staining properties were in accordance with the diagnosis of PVP deposition. The patient had been injecting both buprenorphine tablets and a specific methadone syrup for several years. The methadone syrup contained large amounts of high molecular weight PVP, making it the most likely cause of the deposition. His health quickly deteriorated and he died, impaired by multi-organ failure and cachexia, five years after the first diagnosis of

Open Peer Review

Approval Status ✓ ✓

	1	2
version 2 (revision) 07 Jul 2021	✓ view	
version 1 19 Apr 2021	? view	✓ view

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Any reports and responses or comments on the article can be found at the end of the article.

PVP-deposition. The autopsy revealed extensive PVP-deposition in all sampled organs and tissues.
Conclusions: Histological investigation and the correct identification of PVP in the biopsies led to the discovery of a severe adverse effect from long-standing misuse of a drug. The disseminated PVP deposition likely contributed to multi-organ dysfunction and cachexia with a fatal outcome. The deposited PVP likely originated from repeated injections of a certain methadone syrup.

Keywords
Polyvinylpyrrolidone, PVP, povidone, opioid substitution therapy, opioid substitution drugs, methadone, adverse effect, case report

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REVISED **Amendments from Version 1**

Based on the review reports from the reviewers, we have revised sections of the text and added a reference.

Key words: Case report removed. Foreign material and histiocytic storage added.

Result section:
Under the sub-heading "Biopsy findings": We have added "in Congo red stain or any other stain." to the last sentence.
Under the sub-heading "Further development": We have added a sentence about thrombocytopenia and leukocytopenia.
Under the subheading "Autopsy findings": We have added a sentence about the absence of symptoms of cardiac dysfunction. Sentences about findings in the pancreatic tissue altered for clarification. Sentence added about glomerular findings. Sentence added about signs of acute inflammation. For clarification, we have made a minor revision to the legend for Figure 4.

Discussion section:
In the second paragraph of the discussion: We have added a sentence about differentiating PVP deposits from deposits of crospovidone. New reference added: Ganesan S, Felo J, Saldana M, Kalasinsky VF, Lewin-Smith MR, Tomashefski JF, Jr. (2003) Embolized crospovidone (poly[N-vinyl-2-pyrrolidone]) in the lungs of intravenous drug users. Mod Pathol 16(4):286-92. https://doi.org/10.1097/01.mp.0000062653.65441.da

Any further responses from the reviewers can be found at the end of the article

Background

Injection of oral opioid substitution drugs (OSD) is a concern in the treatment of opioid dependency. An Australian study found that 7–13% of clients in opioid substitution therapy (OST) injected their medication weekly or more often.[1] The OSDs may also be sold on the illegal drug market, and 26.6% of out-of-treatment intravenous drug users in a Norwegian study reported having injected methadone during the past 4 weeks.[2] Injection of oral or sublingual drug formulations may lead to vascular and soft tissue damage with a range of secondary complications.[3]

Several OSDs contain the excipient polyvinylpyrrolidone (PVP),[4] a water-soluble polymer with a wide variety of applications in the pharmaceutical industry.[5] When orally ingested, PVP is not absorbed, and causes no harm.[6,7] When injected, PVP is not metabolized, and the only way of excretion is via glomerular filtration.[6] While low molecular weight (MW) PVP is freely filtered by the glomerulus, PVP with moderate or high MW will be partly or completely retained in the body.[6,8] In the middle part of the last century, PVP was utilized as a plasma expander[9] and as a retarding agent in hormone preparations for injection.[10] Reports from this time described storage in multiple tissues following repeated parenteral administrations of PVP-containing preparations.[9–11]

We report a case of extensive PVP-deposition disease with a fatal outcome following long-term injection of PVP-containing OSDs.

Case presentation

First admission

A male, 30-year-old drug addicted patient in OST was admitted to the local hospital. He was hepatitis C positive and had a history of hospitalisations for skin infection. At admittance, he was in a poor general condition with nausea, vomiting, abdominal pain and muscle aches. Physical examination revealed a diffusely tender abdomen and poor dental status. Laboratory investigations disclosed non-specific inflammatory signs with an increased erythrocyte sedimentation rate (83 mm/h) and C-reactive protein (CRP, 90 mg/L), severe normocytic anaemia with a haemoglobin of 7.8 g/dL and renal insufficiency (serum creatinine 133 µmol/L, estimated glomerular filtration rate 60.1 mL/min/1.73m^2) with microalbuminuria. Blood cultures were negative. Radiological examinations of the thorax and abdomen showed splenomegaly and a pancreatic cyst, but otherwise no radiologic signs of infection, malignancy or kidney pathology. His CRP and serum creatinine levels fell spontaneously, and he left the hospital against the doctor's advice after four days.

Second admission

Two months later, the patient fractured his right clavicle. After a two-week delay, he was admitted to hospital with a fever and a swollen and erythematous clavicular region. His general condition and nutritional status had worsened, nausea and vomiting persisted and anaemia and renal insufficiency had relapsed. Blood cultures were positive for Staphylococcus aureus, and antibiotic treatment against suspected osteomyelitis was initiated. Magnetic resonance imaging (MRI) of the clavicular region and the upper arm showed mottled signal changes with a high signal intensity in the lateral clavicle, the humeral bone and the acromion (Figure 1a). The diagnoses considered at this time were osteomyelitis or malignancy. Biopsies from the fractured bone, bone marrow and gastric mucosa were performed in the work-up of this complex symptomatology.

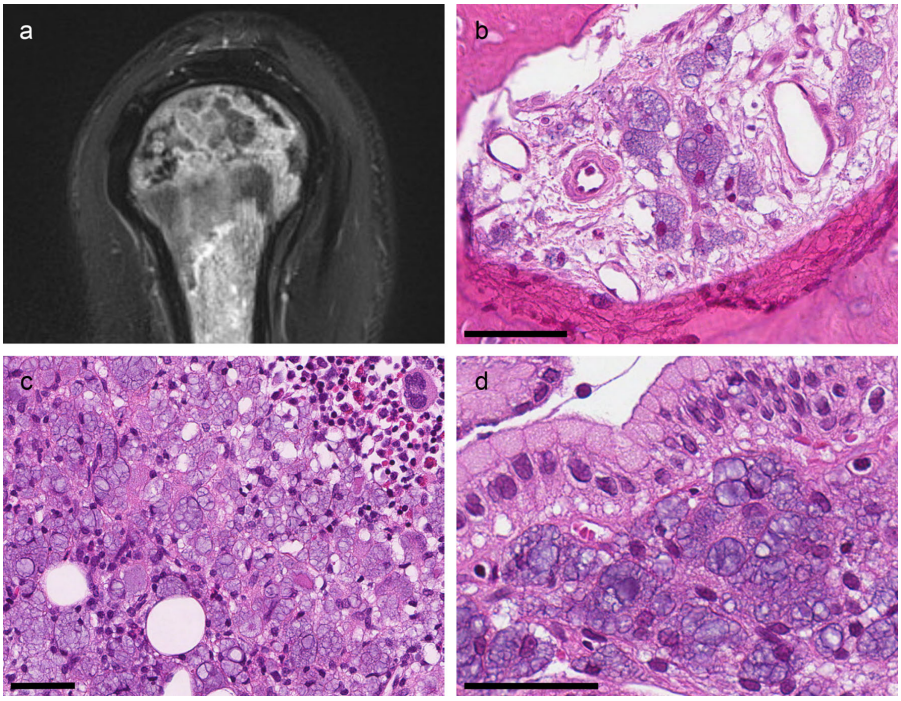

Figure 1. Radiological and biopsy findings. (a) MRI (T2-blade-sag-FS) of the right humerus showing mottled signal changes. (b) Clavicular bone (H&E): The marrow space is infiltrated by histiocytes with bluish transparent bubbles. (c) Bone marrow (H&E): Massive infiltration of histiocytes and scarce remaining hematopoietic tissue. (d) Gastric mucosa (H&E): Infiltration of vacuolated histiocytes in an extended lamina propria. All scale-bars 50 μm.

Biopsy findings

The biopsies all showed similar infiltrates of histiocytes with a cytoplasm extended by vacuoles of different sizes (Figure 1b–d) and eccentrically located nuclei.

Biopsies from the fractured bone revealed reactive changes with ongoing fibrosis. The fibrotic tissue contained the multivacuolated histiocytes as singular cells, small groups or sheets of cells (Figure 1b). The bone marrow biopsy showed massive histiocytic infiltrates (Figure 1c). There was reduced fat cell content, and there was almost no visible hematopoietic tissue. The gastric biopsy showed antrum mucosa with elongated gastric pits and aggregates of multivacuolated histiocytes in both the superficial and deep lamina propria (Figure 1d). A gastric biopsy taken two years previously was re-examined. It showed the same histiocytic infiltrates; however, the findings did not lead to the correct diagnosis at the time.

In the hematoxylin and eosin (H&E) stain, the vacuoles had distinct membranes and a bluish, transparent looking content. Vacuole content did not stain in the periodic acid Schiff (PAS), Prussian blue or Alcian blue stain. The cells were positive for CD68/PGM1 confirming the histiocytic nature (Figure 2a). The vacuoles stained red in Congo red stain and black in the periodic acid methenamine silver (PASM) stain (Figure 2b and c). The vacuoles did not show birefringence in Congo red stain or other stains.

Interpretation

The microscopic appearance of the vacuolated histiocytes and the histochemical staining properties were consistent with PVP deposition.[12,13] At that time, several of the OSDs marketed in Norway contained PVP.[1] Our patient had injected both buprenorphine tablets and methadone syrup regularly over several years. The buprenorphine tablets contained PVP K30 (MW 44–54 kDa). The specific methadone syrup he had been injecting contained large amounts of PVP K90 (MW 1 000–1 500 kDa).[14] While much of the PVP K30 is expected to be excreted within weeks, PVP K90 will not be excreted and

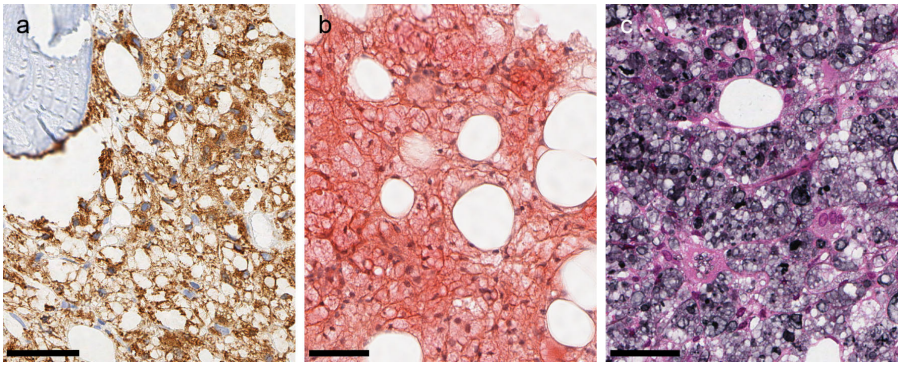

Figure 2. Staining properties. All micrographs are from the same bone marrow biopsy. (a) CD 68/PGM1: The vacuolated cells are CD68-positive histiocytes. (b): Congo red stain: Vacuole content stains faintly red. (b) PASM: Vacuole content stains grey or black. All scale bars 50 μm.

consequently accumulates in the body.[6] It is therefore plausible that injections of the PVP K90-containing methadone syrup were the cause of the PVP-deposition disease in this patient. Based on the history of this patient and other similar cases,[15,16] the European Medicines Agency suspended this methadone syrup in 2014.[14]

Further development

After the diagnosis was made, the patient's health gradually declined, in part due to his poor self-care and underlying chronic drug addiction. There is no specific treatment for PVP deposition disease. His persistent anaemia was treated with regular blood transfusions and erythropoietin-stimulating agents with limited effect. He had short episodes of mild thrombocytopenia and leukocytopenia, and, in general, his leukocyte response to infection was weak. The kidney failure was managed by supportive treatment, and serum creatinine levels fluctuated between 150 and 450 μmol/L.

Within the first year of the diagnosis, the patient suffered a left sided pathological femoral neck fracture with impaired fracture healing, complicated by chronic osteomyelitis. In the right hip, he developed severe bone destruction of the acetabulum with dislocation of the femoral head and a subsequent femoral neck fracture (Figure 3a). MRI scans of both hips and the pelvis (not shown) revealed the same changes as those detected in the shoulder region. Biopsies from the

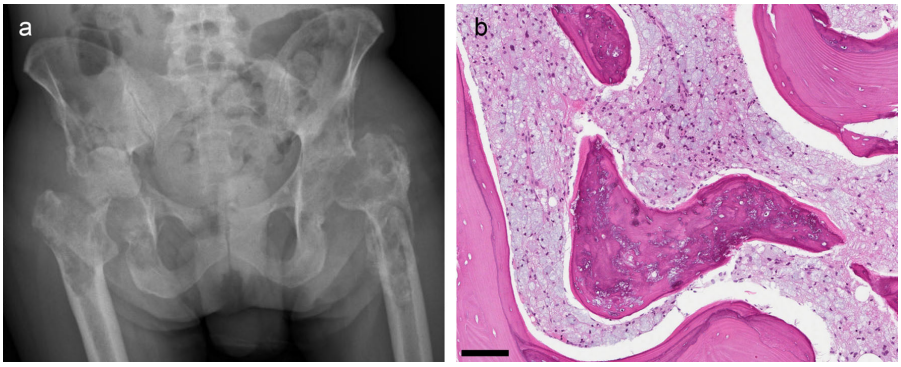

Figure 3. Pathological fractures and bone destruction: radiological and biopsy findings. (a) Pelvic radiograph. Left hip: The hip prosthesis has been removed due to loosening and replaced by a Girdlestone hip. Right hip: Extensive lytic and sclerotic changes in the proximal femur and acetabulum leading to medial dislocation of the femoral head. (b) Biopsy from the right greater trochanter (H&E): The marrow space is filled with histiocytes with the bluish vacuoles characteristic of PVP-deposition. The bone trabecula has empty lacunar spaces and contains a bluish material. Scale bar 50 μm.

greater trochanter region showed extensive histiocytic infiltrates (Figure 3b). Surgical treatments were unsuccessful. Henceforth his mobility deteriorated to such a degree that he needed a wheelchair and required daily activity support and care in a palliative care centre. Furthermore, he developed endocrine pancreas insufficiency with insulin-dependent diabetes mellitus. He had a gradual weight loss of 22 kg during four years despite adequate food intake. Malabsorption due to exocrine pancreas insufficiency was suspected, but supplementation of pancreas enzymes had little effect on the weight loss.

Five years after the diagnosis of PVP deposition disease, the patient died, impaired by multi-organ failure and advanced cachexia with a body mass index below 15 kg/m^2.

Autopsy

The autopsy concluded that the immediate cause of death was multi organ failure due to PVP deposition disease, which in turn was a consequence of the patient's illicit substance abuse. The following organs and tissues were sampled during the autopsy: the pericardium and myocardium, the pleura and lungs, kidneys, liver, pancreas, gastric mucosa, adrenal glands, peritoneum and abdominal adipose tissue, the spleen, lymph nodes, and femoral bone and bone marrow. Microscopically, we observed PVP-deposition in all sampled organs and tissues (Figure 4). The PVP-containing histiocytes were partly scattered in the interstitium, e.g. in the myocardium (Figure 4a), and partly organised like larger, nodular lesions, e.g. in the pleura (Figure 4b). Peri- and intra-neural distribution was seen in several organs including the heart. However, there were no clinical, echocardiographic or electocardiographic evidence of impaired cardiac output or arrhythmias prior to his death. Autopsy findings corresponded well with the clinically observed pancreas and kidney insufficiency. There was little preserved exocrine and endocrine pancreas parenchyma. The pancreatic tissue was dominated by dense fibrosis interspersed by heavy infiltrations of histiocytes (Figure 4c). The kidney showed moderate to severe interstitial fibrosis and tubular atrophy, and only minor glomerular changes. There were infiltrates of PVP-containing histiocytes in the interstitium, mostly in atrophic areas (Figure 4d). In glomeruli, we observed a slightly increased number of histiocytes and occasional PVP-containing vacuoles. There were no amyloid depositions or signs of

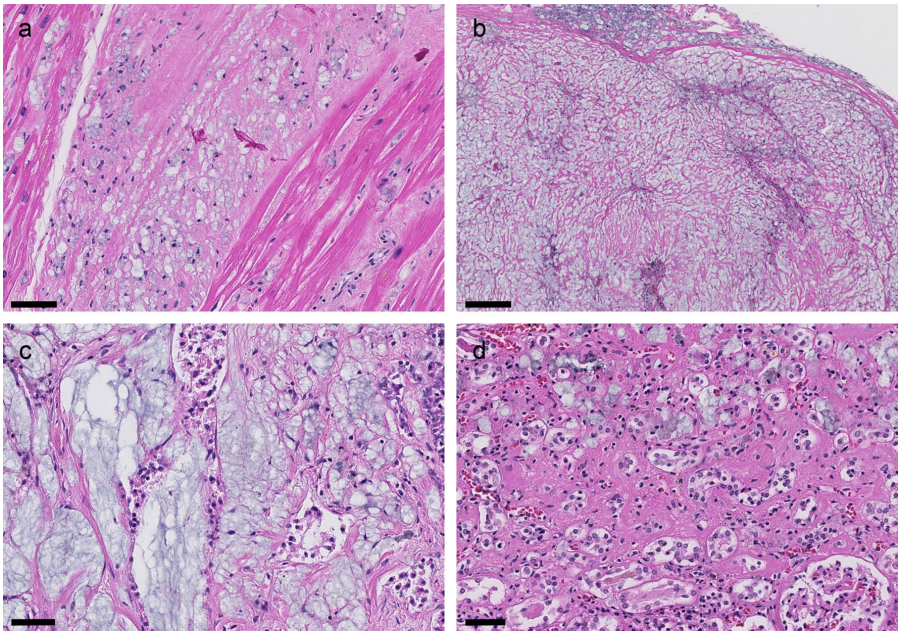

Figure 4. Autopsy findings (H&E). Infiltrates of histiocytes with the bluish vacuoles characteristic of PVP in all organs. (a) Myocardium: Interstitial fibrosis and histiocytic infiltrates. Scale bar 50 μm. (b) Pleura: Nodular lesion composed of fibrotic tissue and histiocytes. We found similar lesions in the pericardium and peritoneum. These were macroscopically visible. Scale bar 200 μm. (c) Pancreas: Pronounced fibrosis with histiocytic infiltrates. Poorly preserved ductal structures. Scale bar 50 μm. (d) Kidney: Cortical tissue showing interstitial fibrosis, tubular atrophy, and an infiltrate of histiocytes in the extended interstitium. Scale bar 50 μm.

other renal diseases. The autopsy revealed no evidence of infection besides minor foci of acute inflammation in the pancreas.

Discussion

We present the case of a drug-addicted patient with widespread PVP-deposition disease. We describe the patient's clinical course from the first diagnosis of PVP-deposition to his death five years later and present biopsy and autopsy findings. The PVP deposition disease in this patient was likely caused by repeated intravenous injections of a methadone syrup containing high MW PVP.

The characteristic appearance and staining properties of PVP in tissue samples are well known.[17] Positivity for Congo red and PASM and negativity for PAS distinguishes PVP deposits from those seen in hereditary storage diseases. The histiocytic nature of the cells and the PAS negativity rule out metastatic signet ring cell carcinoma as a differential diagnosis.[17] PVPs light blue color in H&E and the typical localization of the deposits differentiates it from the non-water soluble variant of PVP, crospovidone.[18] Furthermore, PVP is not birefringent, unlike most other foreign materials commonly observed in tissue samples from injecting drug users.[19] Hence, making the diagnosis of PVP deposition is straightforward if this option is considered.

Whether PVP deposition causes disease was controversial for a long time. The first reports of PVP- deposition described storage in the tissue that persisted years after the administration of PVP, but disputed whether the storage was harmful to the functioning of the target organs.[20] Later reports described clinically relevant adverse effects. Those most frequently reported were cytopenias, bone destruction, polyneuropathy and granulomatous lesions of the skin.[17,21–24] PVP deposition in internal organs such as the liver, kidneys, pancreas and the gastrointestinal tract has also been described, but the adverse effect of the deposition in these organs is less well known.[25,26]

Our patient experienced a complex clinical syndrome and rapidly deteriorating health. The reason for his health decline was multifactorial, and his continued drug addiction likely aggravated the clinical course. Many of the clinical conditions associated with the fatal outcome correspond to findings in biopsies and the autopsy showing extensive PVP deposition. We believe that the extensive PVP deposition contributed to his health decline through several mechanisms. The widespread PVP deposition in bone and bone marrow was likely the main reason for the patient's anaemia, pathological fractures and impaired fracture healing, fitting well with previous reports.[21,23,24] The impaired mobility and chronic osteomyelitis that resulted from these fractures gravely affected his health and quality of life. Furthermore, progressive cachexia was an important part of his health decline. The reason for the weight loss was not established during his lifetime, but his continued drug use likely contributed. Other possible contributing factors were pancreas insufficiency, progressive kidney failure, malabsorption, chronic infections and continued problems with vomiting, all likely related to the extensive PVP deposition. In summary, the PVP deposition probably played a major role in causing the patients' multiple organ dysfunction ultimately leading to the fatal outcome.

Injection of oral OSDs is common among injecting drug users and has long been a concern in the treatment of opioid addiction.[2] As an attempt to prevent injections, the previously mentioned methadone syrup was made highly viscous.[14] However, the increased viscosity did not prevent such unintended use. As a consequence, the choice of high MW PVP as thickener caused further severe adverse effects from injection.

Conclusions

This case revealed an unanticipated explanation for anaemia and pathological fractures in a drug-addicted patient. The correct identification of the observed foreign material as PVP revealed that injection of a certain methadone syrup containing PVP probably caused the patient's deposition disease. Based on the clinical history, biopsy and autopsy findings, we conclude that the widespread PVP deposition likely contributed to the patient's severe morbidity and death.

List of abbreviations

PVP: Polyvinylpyrrolidone

OSD: Opioid substitution drug

OST: Opioid substitution therapy

H&E: Hematoxylin and eosin stain

PASM: Periodic acid methenamine silver stain

PAS: Periodic acid Schiff stain

CRP: C-reactive protein

MRI: Magnetic resonance imaging

MW: Molecular weight

Data availability

All data underlying the results are available as part of the article and no additional source data are required.

Reporting guidelines

Zenodo: CARE checklist for "Polyvinylpyrrolidone deposition disease from repeated injection of opioid substitution drugs: report of a case with a fatal outcome". https://doi.org/10.5281/zenodo.4667989.[27]

Consent for publication

Written informed consent for publication of the patient's clinical details and clinical images was obtained from the patient prior to his death and from the closest relative.

Acknowledgements

We appreciate the contributions of the patient and his family in allowing us to discuss his care and findings. We thank Ingrid Vallevik and Stine Kristoffersen for performing the autopsy and Anna Emilia Kozak for valuable comments on the radiologic images.

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Publisher Full Text

Open Peer Review

Current Peer Review Status: ✔ ✔

Version 2

Reviewer Report 08 July 2021

https://doi.org/10.5256/f1000research.58229.r89200

✔ **Maike Büttner-Herold**
Department of Nephropathology, Institute of Pathology, Friedrich-Alexander University Erlangen-Nuremberg (FAU), Erlangen, Germany

The authors addressed all points I had to my perfect satisfaction and I have no further questions. Thank you for the opportunity to review this very interesting case.

Competing Interests: Maike Büttner-Herold is involved in an European initiative working on a harmonized Kidney biopsy coding (KBC) system, which is coordinated by S. Leh. It is a project in which a large number of European Nephropathologists are involved discussing in meetings of how to best construct a coding system. From time to time we also have Zoom-meetings or email correspondence. This, however, has nothing to do with the case report submitted.

Reviewer Expertise: Nephropathology, GvHD, immunology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 26 May 2021

https://doi.org/10.5256/f1000research.55139.r85062

✔

Robert Barnes Colvin
Department of Pathology, Massachusetts General Hospital, Boston, MA, USA

The paper shows no new information, but the problem is a major one, with missed diagnoses and serious consequences. I think the paper will receive some attention with the major opioid crisis we have.

I have no specific suggestions, other than a careful editing of the prose and the addition of at least on other major publication which has embolized material (EM) - e.g., Ganesan *et al.* (2003)[1].

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Is the background of the case's history and progression described in sufficient detail?
Yes

Are enough details provided of any physical examination and diagnostic tests, treatment given and outcomes?
Yes

Is sufficient discussion included of the importance of the findings and their relevance to future understanding of disease processes, diagnosis or treatment?
Yes

Is the case presented with sufficient detail to be useful for other practitioners?
Yes

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 28 Jun 2021

Ida Viken Stalund, Haukeland University Hospital, Post box 1, 5021 Bergen, Norway

We thank you for your thorough review of our manuscript.

To address your comment, we have added the following sentence in the second paragraph of the discussion: "PVPs light blue color in H&E and the typical localization of the deposits differentiates it from the non-water soluble variant of PVP, crospovidone." and added your suggested reference.

Competing Interests: No competing interests were disclosed.

Reviewer Report 28 April 2021

https://doi.org/10.5256/f1000research.55139.r83519

?

Maike Büttner-Herold
Department of Nephropathology, Institute of Pathology, Friedrich-Alexander University Erlangen-Nuremberg (FAU), Erlangen, Germany

The present case report illustrates a very impressive case of PVP deposition in histiocytes in a large number of organs in a patient who repeatedly injected oral opioid substitution drugs. The description of such a finding is very valuable as the identification of foreign material can be very difficult for a pathologist, if the histological picture has not previously been seen. The illustration of the findings is very nicely done.

Some minor points could be addressed:

- Besides the reported anaemia did the patient also have thrombopenia and leukopenia, which could explain the infectious complications?
- Was the patient free of infectious complications when he died?
- Can a loss of pancreatic islets be shown by immunhohistochemistry? In Figure 4C one wonders whether remaining islets are depicted.
- As PVP is filtered in the glomeruli, were histiocytes detecable in the glomeruli? Was the distribution of PVP-loaden histiocytes in the kidneys dependent on the renal compartment?
- Did the cardiac infiltration by histiocytes lead to functional impairment of the cardiac output or to arrhythmias?
- Is their a possible explanation why PVP should interfer with the structure of the bone in such a destructive way?
- Does the lack of birefringence of PVP also apply to the Congo red staining?
- Keywords: It would maybe be helpful to include "foreign material" and "histiocytic storage" to facilitate search for a pathologist finding PVP in a patient without being able to assign the finding to the right cause.

Is the background of the case's history and progression described in sufficient detail?
Yes

Are enough details provided of any physical examination and diagnostic tests, treatment given and outcomes?

Partly

Is sufficient discussion included of the importance of the findings and their relevance to future understanding of disease processes, diagnosis or treatment?
Yes

Is the case presented with sufficient detail to be useful for other practitioners?
Yes

Competing Interests: Maike Büttner-Herold is involved in an European initiative working on a harmonized Kidney biopsy coding (KBC) system, which is coordinated by S. Leh. It is a project in which a large number of European Nephropathologists are involved discussing in meetings of how to best construct a coding system. From time to time we also have Zoom-meetings or email correspondence. This, however, has nothing to do with the case report submitted.

Reviewer Expertise: Nephropathology, GvHD, immunology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 28 Jun 2021

Ida Viken Stalund, Haukeland University Hospital, Post box 1, 5021 Bergen, Norway

We thank you for your thorough review of our manuscript. We have addressed your comments point by point.

Besides the reported anemia, did the patient also have thrombocytopenia and leukopenia, which could explain the infectious complications?

- The patient had episodes of mild thrombocytopenia and leukopenia. In general, his leukocyte response to infection was weak. We added the following sentence in the section “Further development” to address this question: “He had short episodes of mild thrombocytopenia and leukocytopenia, and in general, his leukocyte response to infection was weak.”

Was the patient free of infectious complications when he died?

- In the days and weeks prior to his death, he had stable, moderately increased CRP. Clinically, he showed no signs of acute infection and he did not receive antibiotic treatment. The autopsy revealed small foci of acute inflammation in the pancreas, but otherwise there were no signs of an acute infection. We added the following sentence in the section “Autopsy” to address this question: "The autopsy revealed no evidence of infection besides minor foci of acute inflammation in the pancreas."

Can a loss of pancreatic islets be shown by immunohistochemistry? In Figure 4C, one wonders whether remaining islets are depicted.

- Based on H&E morphology there was little preserved pancreatic parenchyma, both pancreatic acini and islets. Synaptophysin and Chromogranin A staining revealed a marked loss of pancreatic islets. CK-19 staining revealed areas of some preserved

ductal structures, though the morphology was impaired by autolysis.

We changed the following sentence in the section “Autopsy” to address this question: "There was little preserved exocrine and endocrine pancreas parenchyma. The pancreatic tissue was dominated by dense fibrosis interspersed by heavy infiltrations of histiocytes (Figure 4c)."

- Figure 4C depicts fibrosis, PVP-containing histiocytes and ductal structures impaired by autolysis. Synaptophysin and Chromogranin A staining could not detect islets in this area. We changed the legend to figure 4C accordingly: (c) Pancreas: Pronounced fibrosis with histiocytic infiltrates. Poorly preserved ductal structures.

As PVP is filtered in the glomeruli, were histiocytes detecable in the glomeruli? Was the distribution of PVP-loaden histiocytes in the kidneys dependent on the renal compartment?

- In H&E, we observed occasional PVP-containing vacuoles in glomeruli. With CD68 staining, we detected histiocytes in most glomeruli. The number of histiocytes per glomerulus cross section was variable, but always less than ten. We added the following sentence in the section “Autopsy” to address this question: In glomeruli, we observed a slightly increased number of histiocytes and occasional PVP-containing vacuoles.

- The vast majority of PVP-containing histiocytes were seen in the tubulointerstitial compartment, as stated in the “Autopsy” section.

Did the cardiac infiltration by histiocytes lead to functional impairment of the cardiac output or to arrhythmias?

- The autopsy revealed infiltrates of PVP-containing histiocytes in the myocardium. We also observed peri- and intraneural distribution of the histiocytes. It is possible that such infiltrates could induce arrhythmias or other forms of cardiac impairments. Our patient did not show signs of such organ dysfunction, neither clinically nor in echocardiographic and electrocardiographic evaluations. We added the following sentence in the section “Autopsy” to address this question: “Peri- and intra-neural distribution was seen in several organs, including the heart. However, there were never any clinical, echocardiographic or electrocardiographic evidence of impaired cardiac output or arrhythmias prior to his death.”

Is there a possible explanation why PVP should interfere with the structure of the bone in such a destructive way?

- The only mentioning of a possible cause for the bone destruction is in in a case report from 1993: Kepes *et al.* suggested that cells containing engulfed PVP would undergo mucoid alterations as a result of interactions between the engulfed material and the cytoplasm. The authors argue that in bone, the PVP-induced mucoid changes cause “softening of the bone” and interfere with the structural integrity of the bone trabeculae. One could also speculate that the massive infiltration of histiocytes in the marrow space could affect the blood supply to the osseous tissue, causing a form of avascular necrosis.

We think a discussion of this unresolved matter is out of focus for this case report.

Does the lack of birefringence of PVP also apply to the Congo red staining?

- Yes, the lack of birefringence of PVP also apply to the Congo red. We changed the

following sentence in the section “Biopsy findings” to address this question: “The vacuoles did not show birefringence in Congo red stain or any other stains.”

Keywords: It would maybe be helpful to include "foreign material" and "histiocytic storage" to facilitate search for a pathologist finding PVP in a patient without being able to assign the finding to the right cause

- Good point. We added these keywords.

Competing Interests: No competing interests were disclosed.

II

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Original contribution

Polyvinylpyrrolidone deposition disease in patients with intravenous opioid use: a case series☆,☆☆,☆☆☆

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Summary The polymer polyvinylpyrrolidone (PVP) is an excipient widely used in prescription drugs. Depending on the molecular weight (MW), parenterally administered PVP may accumulate in various tissues. Consequently, moderate and high MW PVP have only been used in oral preparations since the late 1970s. Surprisingly, starting in 2009, pathology departments in Norway received biopsies revealing PVP deposition, all from patients with a history of intravenous drug use. We identified 13 patients with PVP deposition and re-evaluated 31 biopsies and two autopsies. Common indications for biopsy were renal insufficiency, anemia, pathological fractures, and abdominal complaints. We observed PVP deposits in all biopsies (kidney, hematopoietic bone marrow, bone, gastrointestinal tract, lymph node, and skin) and all sampled tissue from the autopsies. Overall, the clinical findings could be related to PVP deposits in the biopsies. In the most seriously affected patients, PVP deposition caused severe organ dysfunction and contributed to the fatal outcomes of two patients. All patients except for one were prescribed opioid substitution drugs (OSDs), and most of the patients admitted to having

Abbreviations: PVP, polyvinylpyrrolidone; MW, molecular weight; OSD, opioid substitution drugs; H&E, hematoxylin and eosin; PASM, periodic acid silver methenamine; IQR, interquartile range; PAS, Periodic acid–Schiff; IFTA, interstitial fibrosis and tubular atrophy.

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injected such medications. Several OSDs contain PVP. One methadone formulation that was marketed in Norway from 2007 to 2014 contained large amounts of very high MW PVP, making it the most likely source of PVP deposition. Although the presumed source of PVP in these patients has now been withdrawn from the market, pathologists should be aware of PVP deposits when evaluating biopsies from this patient group.

1. Background and introduction

Starting in 2009, pathology departments in Norway received an increasing number of diagnostic biopsies showing deposits of polyvinylpyrrolidone (PVP). All patients had a background of opioid addiction, and the majority received opioid substitution therapy.

PVP is a polymer of vinylpyrrolidone. It is widely used as an excipient in tablets and other oral medications [1]. It is neither absorbed from the gastrointestinal (GI) tract nor degraded enzymatically. If injected, elimination is only possible by renal excretion [2]. While PVP of low molecular weight (MW) will be completely excreted, high MW PVP will accumulate in the body's tissues [2]. Over time, and with an increasing dose of PVP, this accumulation can cause clinically relevant organ damage [3]. In the middle part of the last century, PVP was used as a plasma expander and as a retarding agent in injectable hormonal substitution drugs [3]. Because of the discovery of PVP deposition, high MW PVP has not been used in parenteral preparations since the end of the 1970s, at least not in the Western world [3].

Therefore, we were surprised to find PVP deposition in tissue biopsies from 2009 onward. The fact that all patients had a history of opioid addiction and intravenous drug use led us to suspect opioids as a likely source of PVP. The patients had known access to opioid substitution drugs (OSDs). Although OSDs are designed for oral administration, intravenous use is widespread among persons who inject drugs [4,5]. Several OSDs contain PVP [6]. Consequently, these medications represented a possible source for the observed PVP deposition.

The present study has two aims: (1) to describe the clinical and pathological findings related to PVP deposition and increase awareness of this nearly forgotten disease and (2) to explain the occurrence of PVP deposition disease among patients in Norway with a history of intravenous opioid use.

2. Material and methods

2.1. Patients

Patients (n = 13) with PVP deposition were consecutively and retrospectively identified by diagnostic biopsies received at the Department of Pathology at Haukeland University Hospital in Bergen, Norway. The biopsies were received between 2009 and 2013. Clinical data, including drug history, the prescription of OSDs, and laboratory data, were obtained from the referral forms and the patients' medical records. Patient survival was followed until 2020. Two autopsies were available for investigation. The clinical course and findings for one of the included patients were recently published in a case report by the authors [7]. The study was approved by the Regional Committee for Medical and Health Research Ethics (REK 27687).

2.2. Microscopy

Formalin-fixed biopsies and tissue samples from the autopsies were paraffin embedded and sectioned according to standard methods. The diagnosis of PVP deposition was made on hematoxylin and eosin (H&E)-stained sections based on the characteristic histological appearance of the deposited material. In addition, sections with PVP deposits were stained with Congo red and periodic acid silver methenamine (PASM) when required to make the diagnosis with sufficient certainty. Immunohistochemical stains for macrophage markers (CD68 and CD163) were performed on some biopsies.

Kidney biopsies were processed according to standard procedures, including immunohistochemistry for immunoglobulins and complement, as well as ultrastructural investigation of McDowell fixed tissue.

2.3. Statistics and calculations

Statistical analyses were performed using IBM SPSS Statistics version 22. Continuous data are reported using the median (interquartile range [IQR]). To address the second objective, we used previously published data on the distribution of accumulated PVP in the body and data on the rate of elimination of PVP from the body.

3. Results

3.1. Patient characteristics

All 13 patients had a history of intravenous drug use, and all except one received opioid substitution therapy at the time of the first biopsy. The median age was 37 (range

Table 1 Biopsy site, year, and indication for the first biopsy showing PVP deposits for each case. Laboratory parameters are from the time of biopsy. Subsequent biopsies all showed PVP deposition.

ID	Biopsy site	Year	Indication	Hb (g/dL)	WBC (105/L)	TPC (109/L)	eGFR[a]	s−Creatinine (μmol/L)	Subsequent biopsies
1	Kidney	2009	Renal insufficiency	10.9	7.6	329	31	210	−
2	Kidney	2011	Renal insufficiency	10.2	6.7	226	21	275	−
3	Bone marrow	2011	Suspected malignancy	9.0	1.4	37	33	201	−
4[b]	Upper GI tract	2011	Anemia	7.8	5.3	142	54	133	UGI, B x 3, MB, BM, S
5	Kidney	2012	Renal insufficiency	12.6	5.0	241	23	277	−
6	Kidney	2012	Renal insufficiency	9.3	3.0	139	28	220	BM
7	Lower GI tract	2012	Diarrhea	11.4	5.3	224	29	217	BM, K, B
8	Clavicular bone	2012	Pathological bone fracture	9.6	5.6	336	38	172	BM
9	Kidney	2013	Renal insufficiency	11.5	6.5	316	30	170	GB
10	Bone marrow	2013	Suspected malignancy	11.0	4.0	260	24	260	UGI, LGI, LN, K
11	Kidney	2013	Renal insufficiency	11.3	7.6	272	40	168	M
12	Bone marrow	2013	Severe anemia	7.5	3.9	208	9	659	−
13	Skin	2013	Renal insufficiency and suspected PVP deposition	9.7	4.0	76	16	404	−
All patients			Median (IQR)	10.2 (2.2)	5.3 (2.6)	226 (154)	29 (14)	217 (105)	
Reference values				Hb	WBC	TPC		eGFR	s−Creatinine
			Male	13.4 − 17.0	3.5 − 11.0	145 − 348		≥90	60 − 105
			Female	11.7 − 15.3	3.5 − 11.0	145 − 348		≥90	45 − 90

Abbreviations: Hb, hemoglobin; WBC, white blood cell count; TPC, total platelet count; eGFR, estimated glomerular filtration rate; B, bone close to fracture; MB, maxillary bone; BM, bone marrow; S, skin; K, kidney; GB, gall bladder; UGI, upper gastrointestinal tract; LGI, lower gastrointestinal tract; LN, lymph node; M, skeletal muscle.

[a] eGFR (mL/min/1.73 m^2) calculated by the CKD-EPI formula.

[b] Laboratory parameters are from 2 years after the time of the first biopsy.

23−52) years, and 12 of 13 were male. All patients were seropositive for the hepatitis C virus. At the last follow-up in July 2020, six patients (46%) were deceased.

3.2. Clinical and laboratory findings

The main biopsy indications were renal insufficiency (recently discovered), pathological fractures, severe anemia, suspected malignancy, and abdominal complaints (Table 1). In addition, some patients had unspecific complaints, such as skeletal pain, reduced appetite, and weight loss. All patients had increased serum creatinine levels and hemoglobin levels below the reference level (Table 1).

3.3. Biopsy findings

A total of 31 biopsies from the 13 patients were re-examined, and PVP deposits were seen in all tissue samples. The deposits appeared as intracytoplasmic vacuoles in histiocytes. The vacuole content was stained light blue with H&E, red with Congo red stain, gray or black with PASM, and did not stain with Periodic acid−Schiff (PAS) (Fig. 1A−C). The vacuole content was not birefringent in Congo red or other stains. In the ultrastructural examination, vacuole content showed low-to-intermediate electron density with occasional electron-dense granules along the outer limiting membrane (Fig. 1D). Infiltrates of inflammatory cells other than CD 68−positive histiocytes were scarce. In biopsies from living patients, we observed PVP deposits in the kidney, bowel wall, bone marrow, jaws/periodontal tissue, lymph node, and skin.

In biopsies from hematopoietic bone marrow (n = 7), we observed PVP deposits in the marrow space. In four biopsies, the hematopoietic tissue was almost completely displaced by the PVP-containing histiocytes (Fig. 2A and B). In bone samples taken close to fracture sites (n = 5), the PVP deposits also affected necrotic bone tissue (Fig. 2C).

In kidney biopsies (N = 8), PVP deposits were mostly observed in the interstitium of areas affected by interstitial fibrosis and tubular atrophy (IFTA; Figs. 1B and 2D). IFTA was present in all biopsies and widespread in some, whereas glomerular abnormalities were scarce. Ultrastructural examination revealed vacuolated cells both in the interstitium and in the glomerular mesangium.

In samples from the upper and lower GI tract (n = 5), PVP deposits were found in the lamina propria and stretching down into the muscularis mucosae (Figs. 1C and 2E). The epithelial lining was unremarkable.

In a lymph node sample from a patient with lymphadenopathy, PVP deposits were widespread with little remaining lymphoid tissue (Fig. 1A).

Of two skin biopsies, one was performed because of a papular rash and revealed PVP deposits throughout the dermis (Fig. 2F). The second skin biopsy was from a person with renal insufficiency where a kidney biopsy was contraindicated. The biopsy was taken from healthy skin, and the finding of PVP deposits strengthened the suspicion of PVP deposition as the cause for the patients' renal insufficiency.

3.4. Autopsy findings

At the time of the last follow-up in July 2020, six patients were deceased. Two autopsies were available for reinvestigation. The first patient had chronic hepatitis C virus infection with a low viral load. He had a clinical course with months of diminished general health condition, muscle and skeletal pain, and severe weight loss. When admitted to the hospital, pancytopenia, kidney failure, elevated liver enzymes, and a suspected pathological process in the pancreas were uncovered. He deteriorated quickly and died at the end of prolonged hospitalization, presumably from multi-organ failure. The second patient was previously diagnosed with PVP deposition in biopsies from the GI tract, fractured bones, and bone marrow. He died 5 years after the diagnosis was established. During that time, he developed severe and chronic anemia, several pathological fractures, kidney failure, pancreatic failure, and severe cachexia [7].

The two autopsies showed largely similar macroscopic findings. Similarities included enlarged abdominal lymph nodes (Fig. 3A) and firm, white nodular lesions on the pelvic serosa, in the greater omental fold, and the mesentery. In both cases, the pancreas was hardened with a nodular cut surface (Fig. 3A). The pancreatic changes led to a constriction of the distal common bile duct with proximal distension (Fig. 3A). Microscopically, both autopsies revealed infiltrates of PVP-containing histiocytes in all tissue samples (description of each sampled tissue available in Supplementary material A). These included the lungs, heart, liver, pancreas, spleen, kidneys, adrenal glands, mesenteric tissue, lymph nodes, and bone marrow (Fig. 3B–G). The autopsy reports considered acute bronchopneumonia to be the immediate cause of death for the first patient and severe cachexia and multi-organ failure for the second patient. Both indicated widespread and extensive histiocytic infiltrates as part of the chain of events leading to death.

3.5. The source of the PVP deposits

We assessed the amount of PVP that had accumulated in two patients whose bone marrow biopsies appeared nearly full of PVP-containing histiocytes (Fig. 2A and B). To achieve this, we calculated a rough estimate of the total body PVP load based on literature data. Given an average bone marrow mass in male humans of 3000 g [8] and 0.005 g PVP per gram of PVP-saturated tissue [9], the entire bone marrow of these patients contained 15 g PVP. Given that the accumulated PVP in the bone marrow represents 6.5% of the total body load [2], the two patients had accumulated 230 g of PVP.

We then investigated whether injection of OSDs could have caused this level of accumulation of PVP. We studied two PVP-containing OSD preparations marketed in Norway at that time: a methadone syrup containing PVP K90 and buprenorphine tablets containing PVP K30 (information provided by the manufacturer). The calculation showed that patients would have had to inject 392 doses of the methadone syrup or 57,500 doses of buprenorphine

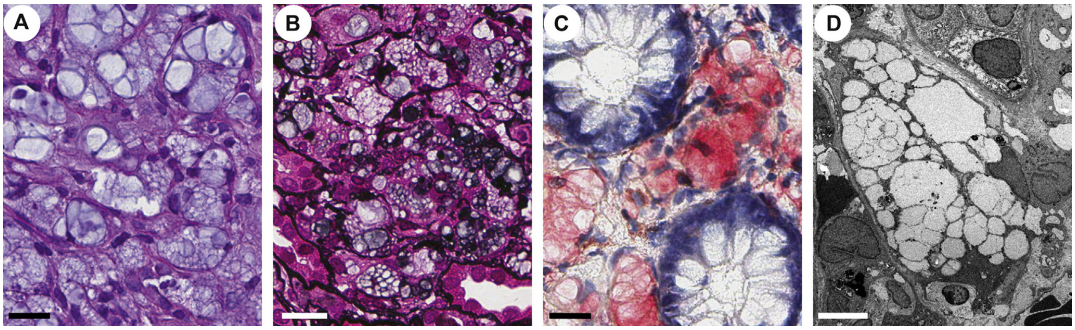

Fig. 1 Staining properties and ultrastructural appearance. (A) Lymph node biopsy. Section stained with PAS. Vacuole content does not stain with PAS and appears gray or light blue. (B) Kidney biopsy. Section stained with PASM. Vacuole content stains gray or black. (C) Biopsy from the lower GI tract. Section stained with Congo red stain. Vacuole content stains weakly to bright red. (D) Kidney biopsy. Ultrastructural investigation. Interstitial histiocyte extended by vacuoles with a low-to-intermediate electron-dense content. Tubular cells in the upper right corner. Scale bars: (A–C) 20 μm. (D) 5 μm. PAS, Periodic acid–Schiff; PASM, periodic acid silver methenamine; GI, gastrointestinal.

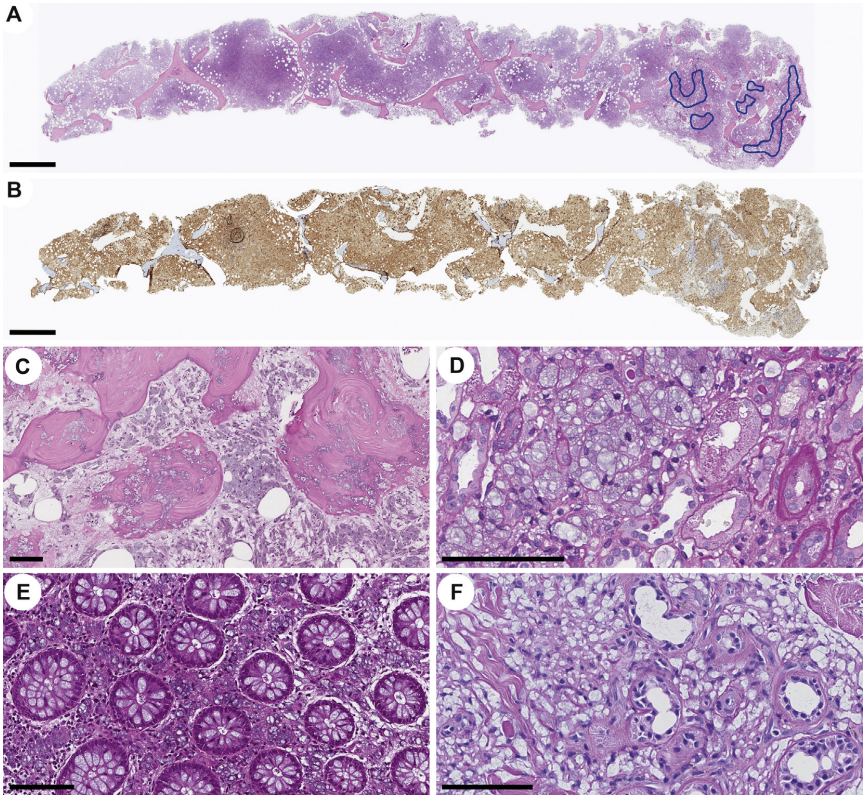

Fig. 2 Biopsy findings showing infiltrates of PVP-containing histiocytes. (A) Iliac crest, H&E: Marrow space is almost completely replaced by the infiltrates of histiocytes. The remaining hematopoietic tissue is annotated in blue. (B) Same biopsy as in Panel A, immunohistochemistry for CD68 (PGM1) illustrating the widespread infiltrates of histiocytes. (c) Bone from femoral head after hip replacement due to femoral head necrosis, H&E. Widespread histiocytic infiltrates in the marrow space. Necrotic bone with osteocytes only in singular lacunae and PVP deposits in many irregular spaces. (D) Kidney, PAS. PVP-containing histiocytes in the expanded interstitium. (E) Lower GI tract, H&E. Crypts surrounded by a slightly extended lamina propria with histiocyte infiltrates. (F) Skin, H&E. Adnexal glands in the deep dermis surrounded by infiltrates of histiocytes. Scale bars: A and B 1000 µm. C–F 100 µm.

tablets to obtain a total PVP body load of 230 g (detailed calculation available in Supplementary material B).

4. Discussion

This case series presents the findings from 31 biopsies and two autopsies from 13 patients with opioid addiction and PVP deposition. A methadone syrup containing large amounts of high MW PVP was the likely source of the bulk of accumulated PVP in these patients. Frequent indications for the biopsies were anemia, pathological fractures, gastrointestinal symptoms, and renal insufficiency. The main finding in most samples was extensive infiltrates of PVP-containing histiocytes, providing an explanation for the clinical symptoms. Of note, even a biopsy of healthy skin contained PVP deposits, which allowed a diagnosis when the intended target organ, the kidney, could not be biopsied. PVP deposition contributed to a fatal outcome in two patients whose autopsies showed PVP deposits in all microscopically investigated tissues.

4.1. The use of PVP in pharmaceuticals

PVP is widely used in the pharmaceutical industry as an excipient [1]. Its pharmacokinetic properties vary with its MW. Of special interest is that the body's ability to excrete PVP diminishes with increasing MW [2].

Low-to-moderate MW PVP was initially used as a colloidal plasma expander during the Second World War [3]. In the following years, there was emerging evidence that a portion of the administered PVP accumulated in the patients' tissues [2]. The observation of PVP accumulation resulted in the abandonment of PVP as a plasma expander in the late 1960s [10]. Meanwhile, the use of moderate-to-

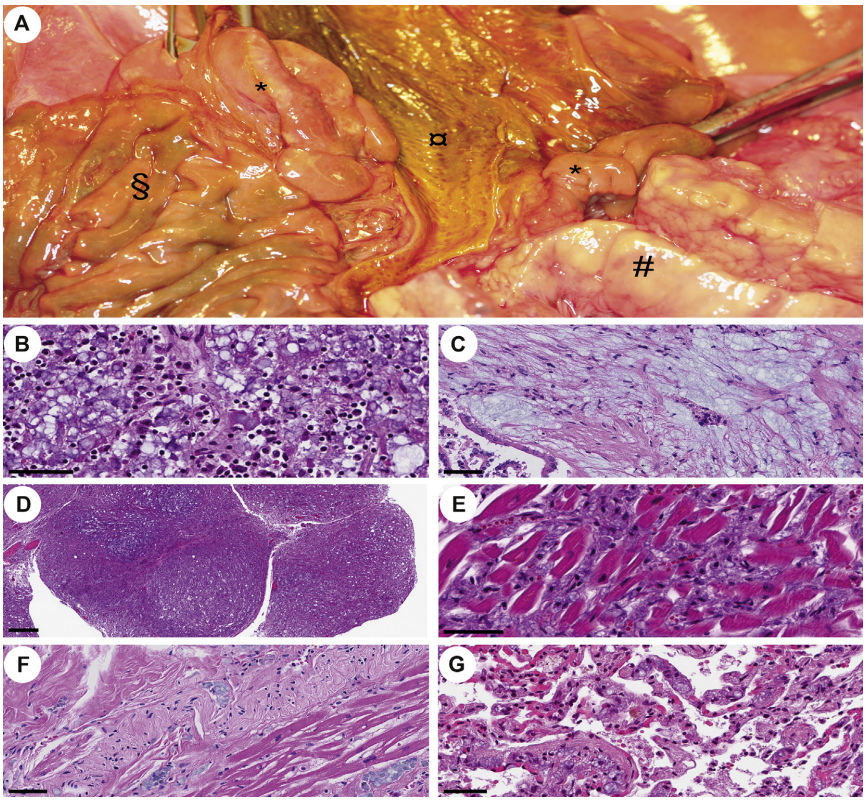

Fig. 3 Autopsy findings. Macroscopy (A) and H&E histology showing PVP-containing histiocytes (B–G). Panels A to F are representative of both autopsies. Findings depicted in Panel G were only seen in the first autopsy. (A) Upper abdominal preparation showing pancreas (#) with a nodular cut surface, constriction of the distal bile duct, and distension of the proximal bile duct (¤). Enlarged lymph nodes (*) on both sides of the bile duct. Duodenal mucosa (§) to the left. (B) Lymph node. Architecture disrupted by dense infiltrates of histiocytes. (C) Pancreas. Extensive fibrosis and infiltration by vacuolated histiocytes. Duct epithelium to the left. (D) Nodular lesion from the mesentery. The lesion is composed of PVP-containing histiocytes and fibrous tissue. (E) Myocardium. Vacuolated histiocytes between unremarkable cardiac myocytes and in areas of interstitial fibrosis. (F) Myocardium. Vacuolated histiocytes with peri- and intra-neural distribution. (G) Lung from the first autopsy. Vacuolated histiocytes in the alveolar walls. Scale bars: D: 300 μm. Otherwise, 50 μm.

high MW PVP as a retarding agent in preparations for daily parenteral administration continued until the late 1970s [3]. At that time, several cases of extensive and clinically relevant PVP deposition from such use of PVP had been reported [9,11]. The most recent reports of PVP deposition were Taiwanese cases resulting from frequent injections of PVP-containing preparations for "nutritional support" or as "blood tonics" [12–16]. The Taiwanese cases resulted in disease manifestations most comparable to the cases in our study.

4.2. Identifying PVP deposits

Recognizing PVP deposits is relatively straightforward, as PVP has distinctive staining properties. It does not stain with PAS, and it stains light blue with H&E, gray or black with PASM, and red with Congo red stain [9]. However, before 2013 when our department became aware that PVP deposition was occurring in patients with opioid addiction, deposits were not recognized at the time of biopsy. The diagnoses considered in the primary evaluation were various histiocytoses, mucin-containing histiocytes, or mucin-producing adenocarcinoma. The misdiagnosis of PVP deposition in the stomach as signet ring cell carcinoma has previously been reported [17]. Other forms of foreign materials may also be considered, as these are common findings in biopsies from patients with intravenous drug use [18]. Unlike many such materials, PVP is not birefringent [9]. Recently, there have been several reports of deposits of crospovidone (the nonsoluble version of

PVP) in tissue samples from persons who inject drugs [19,20]. PVP is easy to distinguish from crospovidone based on the differences in deposition sites and appearance. PVP deposits have been observed in almost all tissues of the body [21,22] and stain light blue in H&E. In contrast, crospovidone deposits are deeply blue, coral-like particles usually observed in the tissue around an injection site or in thromboemboli to the lungs. In addition, crospovidone deposits are often surrounded by a granulomatous reaction [19,20]. In conclusion, PVP deposits are easily identified if the observer is aware of this entity.

4.3. PVP deposition and disease

The biopsies in all our cases were performed because of disease symptoms, clinical findings, and laboratory abnormalities. The infiltrates of vacuolated histiocytes were considered the main finding in most of the biopsies and thus cannot be regarded as incidental. The spectrum of symptoms in our patients was in line with findings reported in the literature, the most common being anemia, pathological fractures, gastrointestinal symptoms, and renal insufficiency.

Anemia was present in all patients. In general, anemia is common among persons who inject drugs [23]. In addition, renal insufficiency, as seen in all our cases, increases the risk of developing anemia [24]. However, in four of the bone marrow biopsies, the PVP deposits almost completely displaced the hematopoietic tissue. Two reports from the 1990s presented similar findings in three patients with severe anemia who had received PVP-containing "blood tonics" [13,14]. The authors concluded, as we do, that the severe anemia was related to the extensive PVP deposits. On the other hand, we have no explanation for the combination of anemia with normal leukocyte and platelet counts in most of our patients.

Three patients suffered from pathological fractures due to osteonecrosis. These findings were in accordance with several previous cases that presented with bone destruction and pathological fractures attributed to PVP deposition [9,13–15]. The bone manifestations are similar to those seen in Gaucher's disease, another storage disease with histiocytosis and osteonecrosis as a major complication [25]. As for Gaucher's disease, hypoperfusion of osseous tissue due to extensive histiocytic infiltrates is one possible explanation for the occurrence of osteonecrosis in patients with PVP deposition.

Gastrointestinal tract biopsies were performed due to abdominal discomfort, vomiting, diarrhea, anemia, and/or severe weight loss. Biopsies revealed PVP deposits in the lamina propria, extending into the muscularis mucosae and the submucosa. Gastrointestinal PVP deposits have previously been described [21]. In another case, abdominal symptoms were attributed to mechanical obstruction by tumoral PVP masses in the mesentery [26]. It is uncertain whether PVP deposits in the bowel wall itself could cause bowel dysfunction and gastrointestinal symptoms. One possible mechanism could be disruption of bowel motility through the involvement of nerves in the bowel wall, as polyneuropathy caused by PVP deposits has been described [9]. Although we were unable to uncover the involvement of nerves in the gastrointestinal samples, we observed peri- and intra-neural distribution of PVP deposits in other organs. The autopsies revealed fibrosis and extensive PVP deposits in the pancreas, bile duct distension, and intra-abdominal tumoral PVP masses, which may have contributed to the symptoms experienced by some of our patients. However, abdominal complaints are common among persons with opioid addiction and are often related to the well-known side effects of opioids [27]. Hence, it was difficult to determine to what extent the gastrointestinal symptoms were related to the PVP deposits.

All patients showed impaired renal function, and kidney biopsies were frequent in our material. The main findings were similar in all biopsies: interstitial infiltrates of PVP-containing histiocytes and IFTA. Persons who inject drugs have an increased risk of developing renal insufficiency, and several mechanisms may lead to IFTA [28]. Because the findings in all biopsies were similar, and most of the changes commonly described in patients who inject drugs were not present [29], we consider PVP-containing histiocytes to be the most likely cause of atrophy and renal insufficiency. Notably, renal insufficiency has only rarely been reported in the setting of PVP deposition [30,31]. This discrepancy may be because of the extremely high MW of the deposited PVP in our cases.

4.4. Autopsies

Our material includes two autopsies. These are valuable, as they yield tissue samples from less accessible organs such as the heart, lungs, and pancreas. We found PVP deposition in all sampled organs. It is often a matter of discussion as to what extent and in which way an autopsy finding contributed to a patient's death. PVP deposits associated with the cardiac conduction systems, as observed in our cases, may induce arrhythmia. Widespread deposits in the parenchyma of the lungs may impair gas exchange. Edelmann et al. and Cabanne et al. described PVP deposition in the lung parenchyma [26,32], which is similar to the findings in one of our cases. The severe involvement of the pancreas in both our cases is puzzling. Kojima et al. observed pancreatic PVP deposits in 11 of 34 cases but did not emphasize this finding [22]. Except for focal acute inflammation in one of our cases, there were no morphological or clinical findings that could explain the widespread occurrence of pancreatic fibrosis. One of these patients had developed endocrine and

exocrine pancreatic insufficiency, which might have exacerbated the severe cachexia that contributed to his death.

There are two autopsy series from the 1960s that presented findings in patients who had received plasma substitutes containing low-to-moderate MW PVP [21,22]. PVP was not considered a causal factor for death except for one patient who died from acute liver failure after more than 2 months of daily plasma substitute infusions [22]. To the best of our knowledge, no other reports describe fatal outcomes from PVP deposition.

4.5. The source of the PVP deposits

A fundamental issue was to identify the source of the PVP deposits in the present cases. In recent years, PVP has mostly been used in oral and topical preparations [1]. Our patients shared similar backgrounds. First, all had a history of opioid addiction and intravenous drug use. Hence, the most likely cause for PVP deposition was the injection of opioids. Second, all except one of the 13 patients received opioid substitution therapy. Injection of OSDs is common among persons who inject drugs [4,5]. In fact, PVP was an excipient in three of the buprenorphine and one of the methadone preparations marketed in Norway [6]. Hence, these drugs were suspected sources. The buprenorphine preparations contained 8 mg of PVP K30 (MW 44−54 kDa) in a normal dose (information provided by the manufacturer). A common dose of the methadone preparation contained 585 mg of PVP K90 (MW 1000−1500 kDa), which was added as a thickener to prevent injection (information provided by the manufacturer). While much of the injected PVP K30 would be excreted within days, weeks, or months, the MW of PVP K90 does not allow renal filtration. Hence, the injected PVP K90 would probably remain in the body [2].

According to our calculations, the most severely affected patients had accumulated about 230 g of PVP. We estimated that it would take the injection of more than 57,500 common buprenorphine doses to reach this level of accumulated PVP, as opposed to less than 400 injections of a common dose of the methadone syrup. Furthermore, the first cases of PVP deposition were seen 2 years after the introduction of this specific methadone syrup to the Norwegian market in 2007. Taken together, it seems highly likely that the methadone preparation containing PVP K90 represented the predominant source of PVP in our cases. Based on the findings presented in this case series, this methadone preparation was withdrawn from the market in Norway and the European Union in 2014 [33]. There are still multiple potentially addictive prescription drugs on the market, which contain low-to-moderate MW PVP [34]. Frequent injection of such drugs may lead to some level of accumulation of PVP, but based on our calculations, we find it unlikely that this accumulation would be sufficient to cause disease.

4.6. Limitations

Chemical analysis can differentiate between PVP K30 and K90 but should be performed on fresh tissue. Unfortunately, we were unable to preserve usable fresh tissue from the autopsies or biopsies.

5. Conclusions

In Norway, persons who inject drugs developed PVP deposition with severe organ dysfunction from the injection of a methadone syrup containing high MW PVP. Our findings demonstrate the importance of documenting morphological changes and identifying foreign materials in tissue samples. Furthermore, pathologists ought to be attentive to signs of adverse effects from injection of oral drugs and should not hesitate to report such findings. Although the presumed source of PVP in these patients has now been withdrawn from the market, pathologists should be aware of PVP deposits when evaluating biopsies from this patient group. Finally, the pharmaceutical industry should bear in mind the risk of unintended parenteral use when designing drugs with addictive properties.

Availability of data and material

All data generated or analyzed during this study are included in this published article.

Code availability

Not applicable.

Authors' contribution

F.L. and I.V.S. made equal contributions to the article and are both considered as first authors. F.L. contributed to conceptualization, investigation, formal analysis, and writing the original article. I.V.S. contributed to investigation, formal analysis, writing the original article, and visualization. T.K.B. contributed to investigation, formal analysis, and reviewing and editing the article. C.O. and E.S. reviewed and edited the article. S.L. contributed to conceptualization, funding acquisition, reviewed and edited the article, and supervision.

Ethics approval

The study was approved by the Regional Committee for Medical and Health Research Ethics (REK), reference number 27687.

Consent to participate

No new information or biological material was gathered on behalf of the study. Because of the social significance of the study, REK approved exemption from the consent requirement for the use of information and biological material from patients gathered in health care services. None of the included patients had registered in the Norwegian Registry of Withdrawal from Biological Research Consent.

Consent for publication

Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humpath.2021.07.009.

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Supplementary material A: Autopsy findings

Article title: Polyvinylpyrrolidone (PVP) deposition disease in patients with intravenous opioid use, a case series

Authors: Friedemann Leh MD, Ida Viken Stalund MD, Tormod Karlsen Bjånes MD PhD, Christian Ohldieck MD, Einar Svarstad MD PhD, Sabine Leh MD PhD

Table A.1 *Microscopic findings in the two autopsies reported in the article*

Organ/tissue	Findings in first autopsy	Findings in second autopsy
Heart	Slight interstitial fibrosis. PVP-deposits in fibrotic tissues, between unremarkable cardiac myocytes and associated with nerve bundles.	Slight interstitial fibrosis. PVP-deposits in fibrotic tissues, between unremarkable cardiac myocytes and associated with nerve bundles. Some nodular lesions in relation to the pericardium.
Lungs	Focal acute inflammation. Widespread PVP-deposits in interlobular septal tissue and in alveolar walls.	PVP-deposits in perivascular spaces and in relation to the pleura, partially in the form of macroscopically visible noduli.
Liver	Periportal fibrosis and some interportal bridges. Portal inflammation scarce. PVP-deposits mainly in portal fields, but also in between hepatocyte plates.	Periportal fibrosis and some interportal bridges. Portal inflammation scarce. PVP-deposits mainly in portal fields, but also in between hepatocyte plates.
Kidney	Largely autolytic tissue. Signs of IFTA. Interstitial PVP-deposits mainly in the corticomedullary border region.	Largely autolytic tissue. Signs of IFTA. Interstitial PVP-deposits. Scattered intratubular oxalate crystals.
Adrenal gland	Not sampled.	Advanced autolysis. PVP-deposits in both cortex and medulla.
Pancreas	Extensive fibrosis with PVP-deposits. Little remaining pancreatic parenchyma. Inflammation scarce besides PVP-containing histiocytes.	Extensive fibrosis with PVP-deposits. Little remaining pancreatic parenchyma. Focal infiltrates of acute inflammation.
Spleen	Advanced autolysis. PVP-deposits in the subcapsular region and scattered diffusely in the parenchyma.	Advanced autolysis. PVP-deposits mainly in the subcapsular region.
Bowel wall	Not sampled.	Advanced autolysis. PVP-deposits in all layers of the wall. Calcifications in relation to the external muscular layer.
Lymph nodes	Widespread PVP-deposits with little remaining lymphatic tissue.	Widespread PVP-deposits with little remaining lymphatic tissue.
Bone marrow	Little remaining hematopoietic tissue. Widespread infiltrates of PVP-containing histiocytes.	Little remaining hematopoietic tissue. Widespread infiltrates of PVP-containing histiocytes. Necrotic bone trabeculae and trabeculae with PVP deposits.
Mesentery	Nodular lesions showing infiltrates of PVP-containing histiocytes surrounded by fibrous tissue.	Not sampled
Pelvic serosa	Nodular lesions of fibrotic tissue and PVP-deposits.	Nodular lesions containing fibrotic tissue and PVP-deposits with focal, circular calcifications.

Both autopsy reports considered the widespread and extensive histiocytic infiltrates with subsequent multi-organ failure as part of the chain of events leading to death. The first of these patients died before our department was perceptive of the phenomenon of PVP deposition caused by injection of OSDs. The autopsy report discussed PVP deposition as a possible differential diagnosis. This thought was discarded, as the patient had not received any PVP-containing plasma substitutes. The report concluded that the immediate cause of death was an acute pneumonia and that multi-organ failure from a non-Langerhans cell histiocytosis was the underlying cause of death. The second autopsy found no signs of acute disease and regarded multi-organ failure and severe cachexia as the immediate cause of death. It concluded that intravenous drug use while in opioid substitution therapy resulting in widespread PVP deposition was the underlying cause of death.

Supplementary material B: Calculation of total body PVP load and the number of injections needed for the observed level of accumulation of PVP

Article title: Polyvinylpyrrolidone (PVP) deposition disease in patients with intravenous opioid use, a case series

Authors: Friedemann Leh MD, Ida Viken Stalund MD, Tormod Karlsen Bjånes MD PhD, Christian Ohldieck MD, Einar Svarstad MD PhD, Sabine Leh MD PhD

Calculation of total body PVP load

Calculations were based on data on average bone marrow mass in male humans (3000 g) [1], PVP load in saturated tissue (0.005 g/g tissue) [2], and the distribution of deposited PVP in different tissue types [3].

Table B.1 *Calculation of total body PVP load in the patients with bone marrow biopsies showing extensive PVP deposition.*

Data	Calculation	Explanation
$0.005\ g\ PVP/g\ tissue$		PVP load in PVP saturated tissue
$3000\ g$		Average bone marrow mass in male humans
	$0.005\ g/g \times 3000g = 15\ g$	PVP mass in bone marrow
$6.5\ \%$		Proportion of bone marrow PVP to total body PVP load
	$(15\ g/6.5) \times 100 = 230\ g$	Estimated total body PVP load

Number of injections needed for the observed accumulation of PVP

Table B.2 *Calculation of the number of injections of common doses of buprenorphine and methadone needed to cause the observed level of accumulation. *We assumed that approximately 50% of injected PVP K30 would remain in the body. The actual value is likely lower. **We assumed that all injected PVP K90 would remain in the body. This assumption is likely accurate.*

Explanation	Buprenorphine tablet	Methadone syrup
Excipient	PVP K30	PVP K90
Mean MW	44 – 54 kDa	1 000 – 1 500 kDa
PVP content per standard dose	8 mg	585 mg
PVP assumed retained per dose	4 mg*	585 mg**
Number of injections needed for accumulation of 230 g PVP	57 500	392

Some of the buprenorphine tablets marketed in Norway contain 8 mg of PVP K30 (mean MW 44 – 54 kDa) in a standard dose [4]. Though the mean MW is low, PVP K30 contains a substantial amount of PVP with a higher MW [5] which is not easily eliminated by glomerular filtration [3]. We assumed that 50 % of the injected PVP K30 would be retained, which is a rather high estimate. A methadone syrup marketed in Scandinavia from 2007 to 2014 contained 585 mg of PVP K90 (mean MW (1000 – 1500 kDa) [4, 6]. This very high molecular weight allows no filtration, and virtually all of the injected PVP would accumulate in the body [3].

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