

A Graphical Overview of the Histopathology of Human Melioidosis: A Case Series

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Background. Melioidosis, caused by the Gram-negative bacterium *Burkholderia pseudomallei*, has a major global health impact and a wide range of different disease manifestations. Histopathological descriptions of melioidosis remain limited. Granulomatous inflammation with multinucleated giant cells are considered classic features. We aim to present a graphical overview of histopathological manifestations of melioidosis, serving as an aid in diagnosing this disease.

Methods. We performed a retrospective international multicenter laboratory-based analysis of formalin-fixed paraffin-embedded (FFPE) tissue from culture-confirmed melioidosis autopsy and biopsy cases. Available FFPE tissue was stained with hematoxylin and eosin and immunostainings including a monoclonal antibody targeting the capsular polysaccharide (CPS) of *B. pseudomallei*. Tissue with site-specific cultures and/or positive CPS staining were included in the graphical histopathological overview.

Results. We identified tissue of 8 autopsy and 5 biopsy cases. Pneumonia and soft tissue abscesses were the leading foci of disease displaying mainly necrosis and suppuration. Infrequent disease manifestations included involvement of bone marrow and adrenal glands in an autopsy case and biopsied mediastinal tissue, the latter being the only case in which we identified multinucleated giant cells. Using the CPS staining, we demonstrated granulomata as part of rare gastric tissue involvement.

Conclusions. We found fatal melioidosis to be a necrotizing and suppurative inflammation, usually without multinucleated giant cell formation. Gastric and mediastinal involvement points to ingestion and inhalation as possible routes of infection. The CPS staining proved beneficial as an aid to establish a histopathological diagnosis. Our graphical overview can be used by infectious diseases specialists, microbiologists, and pathologists.

Keywords. *Burkholderia pseudomallei*; histopathology; melioidosis.

More than a century ago, Whitmore and Krishnaswami [1] described a thus far unknown disease in Burma now known as the tropical infectious disease melioidosis [2]. The Gram-negative facultative intracellular soil and water-dwelling bacterium *Burkholderia pseudomallei* is the causative agent of melioidosis, a disease that is well established in Southeast Asia and northern Australia, although present throughout the tropics [2, 3]. Yearly, modeling predicted that 165 000 people acquire infection, 89 000 of whom die [3]. Despite this enormous global disease burden, melioidosis is not officially recognized as a

neglected tropical disease by the World Health Organization [4, 5]. Due to current global travel and increasing trade, melioidosis is not limited to endemic countries, but it also poses a risk to inhabitants of nonendemic regions [6, 7]. This was recently highlighted by a multistate outbreak of melioidosis in the United States of America involving 4 cases (2 fatal), confirmed by bacterial genomics to be infection attributed to the importation of *B. pseudomallei*-contaminated room spray from India [8].

Melioidosis has been referred to as “the great mimicker” with frequent disease manifestations that include pneumonia or bacteremia, but it can also present as local skin infection, internal organ abscess formation (eg, spleen, liver), and musculoskeletal or neurological involvement, although the latter 2 are less frequently seen [2, 9, 10]. The majority of cases are acute (approximately 88%), the remainder being chronic as defined by symptoms being present for 2 months or longer [2, 4, 9]. Studies from Thailand and Australia together with a systematic review of clinical data of melioidosis patients demonstrated adult males to be predominantly affected [4, 9, 10]. Acquisition of disease occurs through skin inoculation, inhalation/aspiration, or ingestion,

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and predisposing risk factors include diabetes and chronic conditions (eg, liver, kidney, lung), with diabetes present in approximately half of cases [2, 4, 9, 10].

The initial postmortem examinations of Whitmore and Krishnaswami [1] were the first to describe the microbiological and pathological manifestations of the septic and pyogenic aspects of the disease melioidosis. Lung, liver, and kidney displayed soft (“cheesy”) lesions surrounded by hemorrhage, and abscesses were also observed, such as in the spleen. In the recent past, melioidosis has been described in autopsy cases of American soldiers who served in the Vietnam war and was nicknamed the “Vietnam Time Bomb” due to its potential to cause disease years after exposure [11–14]. A report from 1995 systematically described melioidosis histopathology as a necrotizing and/or granulomatous inflammation with abscess formation and multinucleated giant cells [15], thereby resembling tuberculosis. Giant cells have been considered a hallmark of melioidosis [16]. In addition, immunohistochemistry has previously been studied as a means to improve diagnosis of melioidosis, but culture remains the gold standard, especially because melioidosis can be easily misdiagnosed as tuberculosis [2, 16, 17]. Until now, human histopathological data remain limited and consist primarily of historical case series and case reports. We aim to present a graphical overview of histopathological manifestations to be used as an aid in establishing a diagnosis of melioidosis.

METHODS

We performed a retrospective international multicentre laboratory-based analysis of archival formalin-fixed paraffin-embedded (FFPE) tissue. Our case series combined FFPE tissue from culture-confirmed melioidosis cases derived from a retrospective travelers cohort of the Dutch Melioidosis Study Group [7] and the Darwin Prospective Melioidosis Study from the Northern Territory of Australia [9], a highly endemic melioidosis region. We only focussed on available archival FFPE tissue because in some cases, not all possibly affected tissue was available for analysis. Available FFPE tissue of autopsy and biopsy cases with site-specific cultures and/or positive staining directed at *B pseudomallei* was included in the graphical histopathological overview (see below).

Staining and Evaluation of Tissue Sections

Tissue sections were prepared for all available FFPE tissue. All tissue sections were deparaffinized and stained with hematoxylin and eosin or prepared for immunostainings. In short, preparatory steps for the immunostainings included blocking for endogenous peroxidase, heat-induced antigen retrieval, and blocking of nonspecific antibody binding followed by primary antibody binding using the antibodies anti-myeloperoxidase (MPO), anti-CD3, anti-CD20, anti-CD68, anti-CD138, antifibrin(ogen),

and a monoclonal mouse antibody 4C4 targeting the capsular polysaccharide (CPS) of (mainly) *B pseudomallei* and *Burkholderia mallei* (Supplementary Appendix) [18]. The CPS antibodies were kindly provided by David P. AuCoin of the University of Nevada, Reno and validated using affected and unaffected tissue. We used an appropriate horseradish peroxidase labeling system and visualized antigens using 3,3-diaminobenzidine. All tissue sections were counterstained with hematoxylin and evaluated by 2 observers (JS and JJTHR) in a nonblinded fashion.

Inclusion and Exclusion of Cases and/or Tissue Sections

We made sure to report melioidosis-associated histopathological features by using only tissue sections with site-specific positive cultures for *B pseudomallei* and/or positivity on CPS staining. For autopsy cases, tissue sections were only included from patients whose death was confirmed by bacterial culture to be from melioidosis and where there was positivity on CPS staining. For biopsy cases, we specifically did not exclude negatively CPS-stained tissue sections in the presence of site-specific positive cultures because we could not compare the staining patterns with lesions of other organs from the same case, and biopsy cases tend to have less pronounced inflammation.

Ethical Considerations and Patient Consent Statement

The Medical Research Involving Human Subjects Act (WMO) does not apply to this study as reviewed by the Medical Ethics Review Committee of the Academic Medical Center (reference number W20_428 # 20.475). The Darwin Prospective Melioidosis Study received ethical approval of the Human Research Ethics Committee of the Northern Territory Department of Health and Menzies School of Health Research (reference number HREC 02/38). Informed consent was obtained or an exemption for the need of consent was applicable.

RESULTS

We identified 13 culture-confirmed melioidosis cases that contained 8 autopsy and 5 biopsy cases with a majority of males and an age across cases that ranged from 37 to 73 years (Table 1). Overall, pneumonia and soft tissue abscess formation were the leading histopathological manifestations, and we also included tissue of organs less commonly associated with melioidosis (Table 2). The majority of the autopsy cases had environmental exposure and presented as sudden and unexpected deaths with no appropriate supportive and/or antimelioidosis treatment. We report the histopathological findings for autopsy cases as pulmonary and extrapulmonary and show the variety of histopathological manifestations. Reasons for exclusion of tissue sections were tissue that was incorrectly sampled or

Table 1. Demographic and Clinical Details of Culture-Confirmed Melioidosis Cases

Case ID	Sex, Age (Years)	Country of Acquisition ^a	Duration of Symptoms Before Presentation	Foci of Disease ^b	Risk Factors for Melioidosis ^{c,d}	Environmental Exposure	Culture
Autopsy Cases							
C1	Male, 55	Australia	Unknown	Pneumonia with disseminated disease	Hazardous alcohol use	Yes	Postmortem lung
C2	Male, 66	Australia	Unknown	Prostatitis with disseminated disease	Chronic lung disease, hazardous alcohol use	Yes	Postmortem pus swab of prostate gland
C3	Male, 50	Australia	Unknown	Pneumonia with disseminated disease	Diabetes, hazardous alcohol use	Yes	Postmortem lung
C4	Male, 43	Australia	Unknown	Pneumonia and prostatitis with disseminated disease	Hazardous alcohol use	Yes	Postmortem pus swab of prostate gland
C5	Female, 73	Thailand, Australia	5 days	Pneumonia with disseminated disease	None	No	Sputum and blood; postmortem lung
C6	Male, 45	Australia	Unknown	Pneumonia, relapsed melioidosis	Diabetes, chronic lung disease, hazardous alcohol use	Yes	Postmortem lung
C7	Male, 37	Australia	Unknown	Pneumonia with disseminated disease	Diabetes, morbid obesity	No	Postmortem lung
C8	Female, 50	Australia	Unknown	Pneumonia with disseminated disease	Diabetes, chronic kidney disease, ischemic heart disease with dilated cardiomyopathy and extensive myocardial fibrosis	Yes	Postmortem blood, lung, kidney, and mediastinal lymph node
Biopsy Cases							
C9	Male, 40	Australia	Months (unspecified)	Acute abdomen due to gastric ulcer with disseminated disease	Diabetes, hazardous alcohol use	No	Blood, gastric aspirate, abdominal/subphrenic pus, sputum, pleural fluid
C10	Male, 69	Thailand	42 days	Mediastinal lymphadenopathy	None	Yes	Pus lymph node
C11	Male, 63	The Gambia	3 days	Prostatitis with disseminated disease	Chronic kidney disease	Yes	Blood, urine, prostate
C12	Female, 53	Thailand, Vietnam, Indonesia (Bali), Sri Lanka	Unknown	Lung and lymph node abscess	Chronic lung disease	No	Lymph peripheral smear
C13	Male, 73	Thailand	Unknown	SSTI of neck muscle	Diabetes	No	Pus SSTI neck

Abbreviations: ID, identification; SSTI, skin and soft tissue infection.

^aCases were domestically acquired in Australia or traveler-associated with the possibility of multiple visited countries.

^bCases were either confined to their foci of disease or showed features of dissemination.

^cComorbidities not associated with an increased risk of acquiring melioidosis were disregarded.

^dLiver abnormalities were not classified as a separate risk factor because the findings presented as plain steatosis or were consistent with hazardous alcohol use.

showed negativity on the CPS staining, which typically involved sections without infectious abnormalities.

Autopsy Cases: Pulmonary Manifestations

Pulmonary involvement was described in all autopsy cases ($n = 8$) (Figure 1). Lung samples invariably showed signs of (confluent) necrotizing (8 of 8) and suppurative (8 of 8) pneumonia in different stages of organization. Bronchitis was present in all cases (8 of 8), whereas endothelialitis (5 of 8) and pleuritic involvement (2 of 8) were less often seen, although we did not specifically sample pleurae. Granulomas and

multinucleated giant cells were notably absent. Other nonspecific features included (intra-alveolar) edema (7 of 8) and hemorrhage (6 of 8). Thrombosis was scarce (1 of 8) despite staining for fibrin(ogen), which showed nonspecific inflammation-related fibrin depositions. In general, neutrophils were the predominant cell type admixed with varying numbers of macrophages and lymphocytes. Neutrophil extracellular traps were occasionally spotted. Inflammatory infiltrates stained convincingly positive for CPS (Figure 2A and B), both intra- and extracellular, and was largely in line with the MPO staining pattern.

Table 2. Overview of Available Tissue Sections and Histopathological Diagnosis of Culture-Confirmed Melioidosis Cases

Case ID	Sex, Age (Years)	Tissue Sections and Presence of Characteristic Histopathological Findings for Melioidosis ^{a,b}	<i>Burkholderia pseudomallei</i> CPS Staining ^a	Histopathological Diagnosis
Autopsy Cases				
C1	Male, 55	Lung: abscess formation, necrosis Liver: abscess formation Spleen: abscess formation	Positive	Pneumonia with abscess formation in liver and spleen
C2	Male, 66	Lung: abscess formation, necrosis Kidney: abscess formation, necrosis Prostate: abscess formation, necrosis Bladder: abscess formation, necrosis	Positive	Prostatitis with pneumonia and abscess formation in kidney and bladder
C3	Male, 50	Lung: abscess formation, necrosis Liver: abscess formation Spleen: abscess formation	Positive	Pneumonia with abscess formation in liver and spleen
C4	Male, 43	Lung: abscess formation, necrosis Prostate: abscess formation, necrosis	Positive	Pneumonia and prostatitis
C5	Female, 73	Lung: abscess formation, necrosis Liver: abscess formation Spleen: abscess formation, necrosis Bone marrow: abscess formation, necrosis Adrenal gland: abscess formation, necrosis	Positive	Pneumonia with abscess formation in liver, spleen, bone marrow and adrenal gland
C6	Male, 45	Lung: abscess formation, necrosis	Positive	Pneumonia
C7	Male, 37	Lung: abscess formation, necrosis	Positive	Pneumonia
C8	Female, 50	Lung: abscess formation, necrosis	Positive	Pneumonia
Biopsy Cases				
C9	Male, 40	Stomach: abscess formation, necrosis, granulomatous inflammation	Positive	Gastritis
C10	Male, 69	Mediastinal lymph node: necrosis, granulomatous inflammation, multinucleated giant cell formation	Negative	Lymphadenitis
C11	Male, 63	Prostate: abscess formation	Positive	Prostatitis
C12	Female, 53	Lung: abscess formation, necrosis	Positive	Pneumonia
C13	Male, 73	Subcutaneous and muscle: necrosis	Negative	Panniculitis

Abbreviation: ID, identification; CPS, capsular polysaccharide.

^aAll tissue sections of culture-confirmed melioidosis cases had site-specific positive cultures for *B. pseudomallei* and/or stained positive for CPS. For the autopsy cases, only the positive CPS-stained tissue sections are mentioned.

^bCharacteristic histopathological findings for melioidosis include abscess formation, necrosis, granulomatous inflammation, and multinucleated giant cell formation.

Autopsy Cases: Extrapulmonary Manifestations

Extrapulmonary involvement was found in 5 autopsy cases ($n = 5$), and available affected tissue included the liver (3 of 5), spleen (3 of 5), prostate (2 of 5), kidney (1 of 5), bladder (1 of 5), bone marrow (1 of 5), and adrenal gland (1 of 5) (Figure 3). Extrapulmonary involvement invariably showed signs of (micro)abscess formation (5 of 5) or necrotizing inflammation (3 of 5). Multiple microabscesses were mainly present in liver, spleen, adrenal, and bone marrow tissue, whereas extensive suppurative inflammation was observed in the prostate, kidney, and bladder (Figure 2C and D). Of note, in one case with extensive prostatic involvement, the urinary bladder showed signs of cystitis with partial rupture of the inner and outer bladder wall due to severe transmural inflammatory damage (C2). Granulomas and multinucleated giant cells were absent. Less common nonspecific features included hemorrhage (2 of 5), edema (1 of 5), and thrombus formation (1 of 5). The cell types involved were similar to those seen in pulmonary involvement with a predominant neutrophilic component.

Biopsy Cases

Biopsy cases included tissue from lung, lymph node, prostate, subcutaneous/muscle, and stomach (all $n = 1$) (Figure 4). The pattern of inflammation was with diminishing frequency necrotizing (4 of 5), suppurative (3 of 5), and granulomatous (2 of 5). We identified one of our cases with a known history of symptoms before presentation (42 days) to present with granulomatous inflammation and presence of multinucleated giant cells, which was the only case with giant cell formation in our series (C10). Edema (1 of 5), hemorrhage (1 of 5), and thrombi (0 of 5) were not widely present features in biopsy cases. In comparison to the autopsy cases, the predominant inflammatory cell types showed more variation with varying amounts of neutrophils, macrophages/histiocytes, lymphocytes, and plasma cells. Similar to the autopsy cases, fibrin(ogen) staining showed nonspecific inflammation-related fibrin depositions. CPS staining was positive in 3 cases and contributed to the tissue-specific diagnosis in the case with involvement of the stomach (C9) (Figure 4A). The stomach displayed intramural and subserosal necrotizing, suppurative, and granulomatous

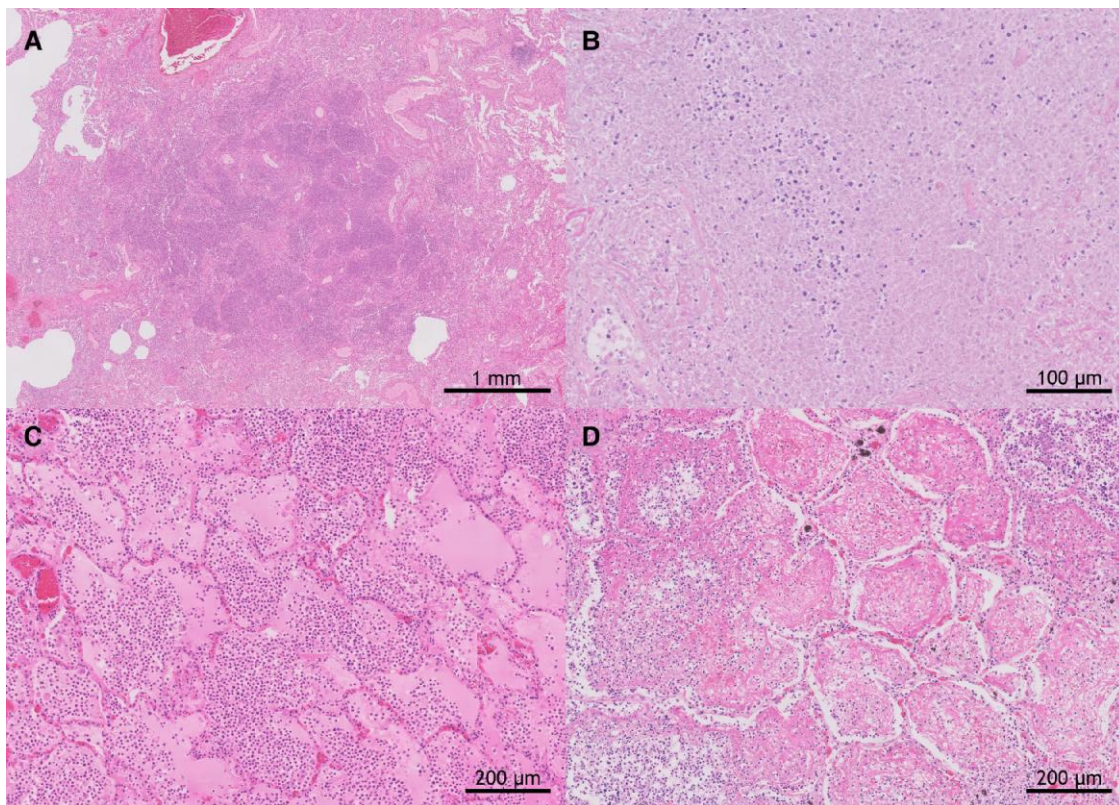


Figure 1. Histopathology of pulmonary manifestations of fatal melioidosis. Images all show hematoxylin and eosin staining. (A) Example as part of a necrotizing and suppurative pneumonia with a highlighted area of an infiltrate in C2. (B) Necrotizing and suppurative pneumonia with area of extensive necrosis that contains minor influx of inflammatory cells in C3. (C) Necrotizing and suppurative pneumonia with area of intra-alveolar edema and hemorrhage in C4. (D) Pneumonia with organizing features in C5.

inflammation, accompanied by a wound healing response, characterized by hemorrhage, edema, granulation tissue, and fibrosis. Neutrophils predominated the mixed cellular infiltrate. The CPS staining was sparsely positive and not associated with the granulomas. Melioidosis with such involvement of the stomach is extremely uncommon.

DISCUSSION

Melioidosis has a major global health impact and a wide range of disease manifestations. Until now, detailed and comprehensive descriptions of the histopathology of human melioidosis remain limited. In this study, we present a graphical overview of the histopathological manifestations, displaying a diverse spectrum of organ involvement in melioidosis.

Pneumonia and intra-abdominal abscesses are among the most common organ-specific entities of melioidosis, and therefore we identified a number of tissues derived from lung, liver, and spleen [2, 9, 10]. Histopathological features previously described include abscess formation, necrotizing and/or granulomatous inflammation, multinucleated giant cells, hemorrhage, and fibrin thrombi [13–15, 19, 20]. In the Results and Discussion sections, we have divided our findings of autopsy

cases into pulmonary and extrapulmonary involvement and make comparisons to a handful of biopsy cases.

Autopsy Cases and the Acute Stages of Disease

Melioidosis presents as an acute illness in the majority of cases [4, 9]. It was not possible to ascertain the duration of symptoms for all autopsy cases because the majority of cases presented as sudden and unexpected deaths, with limited circumstantial information, and no evidence of recent interaction with health-care services related to possible melioidosis. However, given the severity of disease, we considered the cases to represent acute disease. The acute stages of disease in our case series were characterized by suppurative and necrotizing inflammation in all cases and the majority of tissue analyzed.

Our autopsy cases represent severe illness with heavily positive staining for CPS. It is interesting to note that we did not identify any granulomas or multinucleated giant cells in the autopsy cases, the latter being considered as a hallmark of both acute and chronic disease [16]. We therefore presume that formation of multinucleated giant cells occurs during chronic disease processes rather than in fulminant melioidosis sepsis. Similar findings were observed in chronic animal model studies

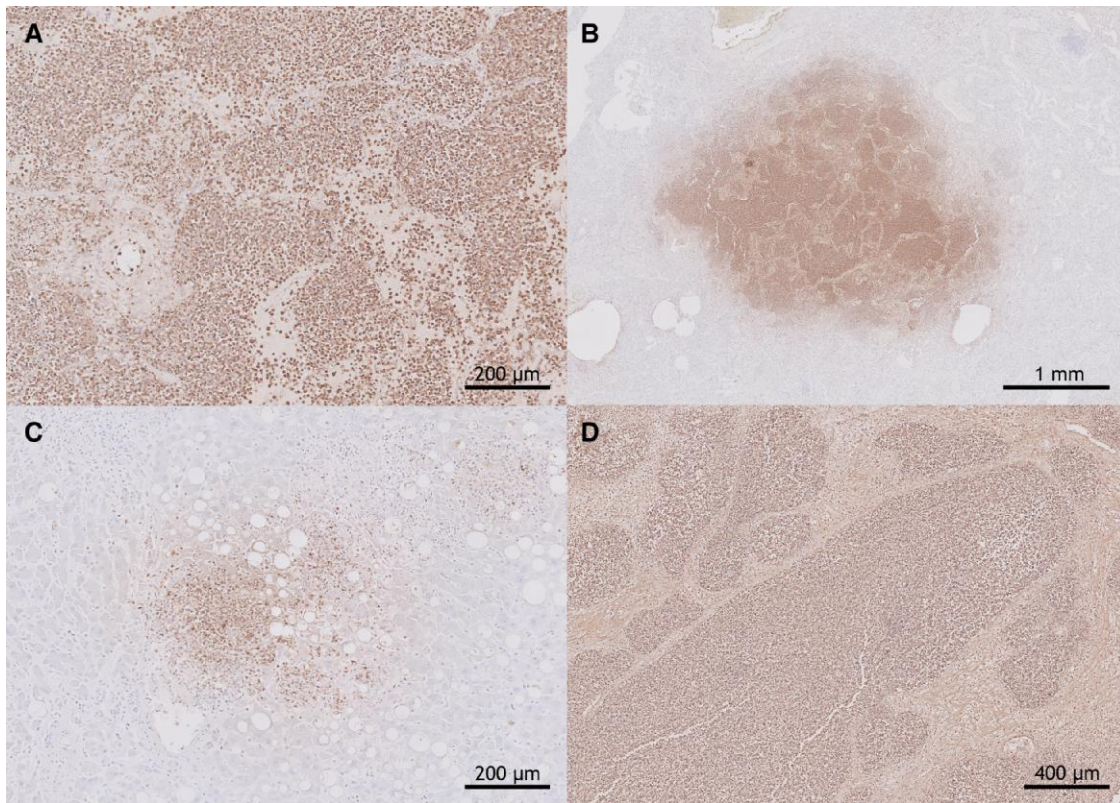


Figure 2. Immunohistochemistry. (A) *B pseudomallei* (*Bps*) staining pattern of a necrotizing and suppurative pneumonia in C1. (B) Another example of a *Bps* staining pattern as part of a necrotizing and suppurative pneumonia in C2. (C) *Bps* staining pattern of a liver abscess in C3. (D) *Bps* staining pattern of an extensive suppurative prostatitis in C4.

(mice, goats) that showed giant cells to be present in later stages of disease, which suggests that time is required for its development [21, 22]. In vitro studies have shown that *B pseudomallei* can induce giant cell formation in phagocytic cell lines within hours after infection, but the clean and controlled culture environment is distinctly different from the complex in vivo situation in humans and other mammals [23, 24]. A human study reported fibrin thrombi to be present in 5 of 6 autopsies, but it highlighted difficulties in its detection [19]. Nevertheless, fibrin thrombi were rarely observed in our case series despite severe disseminated disease of the autopsy cases and the previously described activation of coagulation in melioidosis [25].

Infrequent organ involvement included adrenal gland and bone marrow tissue. The Darwin Prospective Melioidosis Study identified adrenal abscesses in 5 melioidosis cases in a cohort of over 1000 melioidosis patients, and associated features reported in the literature were largely in line with the general histopathological features we observed [9, 12–15, 19]. Bone marrow followed a similar pattern of disease and further features reported in the literature included a hypocellular marrow, hypoplasia with a left shift of granulocytes, and (possible) hemophagocytic lymphohistiocytosis [12–14, 19, 26–28].

Biopsy Cases and Evidence Pointing Towards Inhalation and Ingestion as Route of Infection

Chronic disease in melioidosis is considered a duration of symptoms of 2 months or longer [2], which we were able to identify in one of our biopsy cases (C9). Another case presented with symptoms of 42 days but with a travel history of 7 months ago to Thailand (C10), thereby suggesting a possible chronic disease process [7]. A third case had a symptom duration of 3 days (C11), and symptom duration of the remaining cases remains unknown. It is interesting to note that the 2 cases with a (possible) chronic duration were the only cases that presented with granulomas and multinucleated giant cells, consistent with granulomatous inflammation and multinucleated giant cell formation reflecting more of a chronic disease process; as previously noted, we encountered neither feature in the acute stages of disease in our autopsy series; although another explanation could be the rapid onset and severity of disease and not acute disease in itself. The predominant inflammatory cell types in these cases showed more variation, reflecting the longer disease course in contrast to the acute disease stages in the autopsy cases.

Our biopsy cases provide evidence to suggest inhalation and ingestion as routes of infection as supported by the cases with

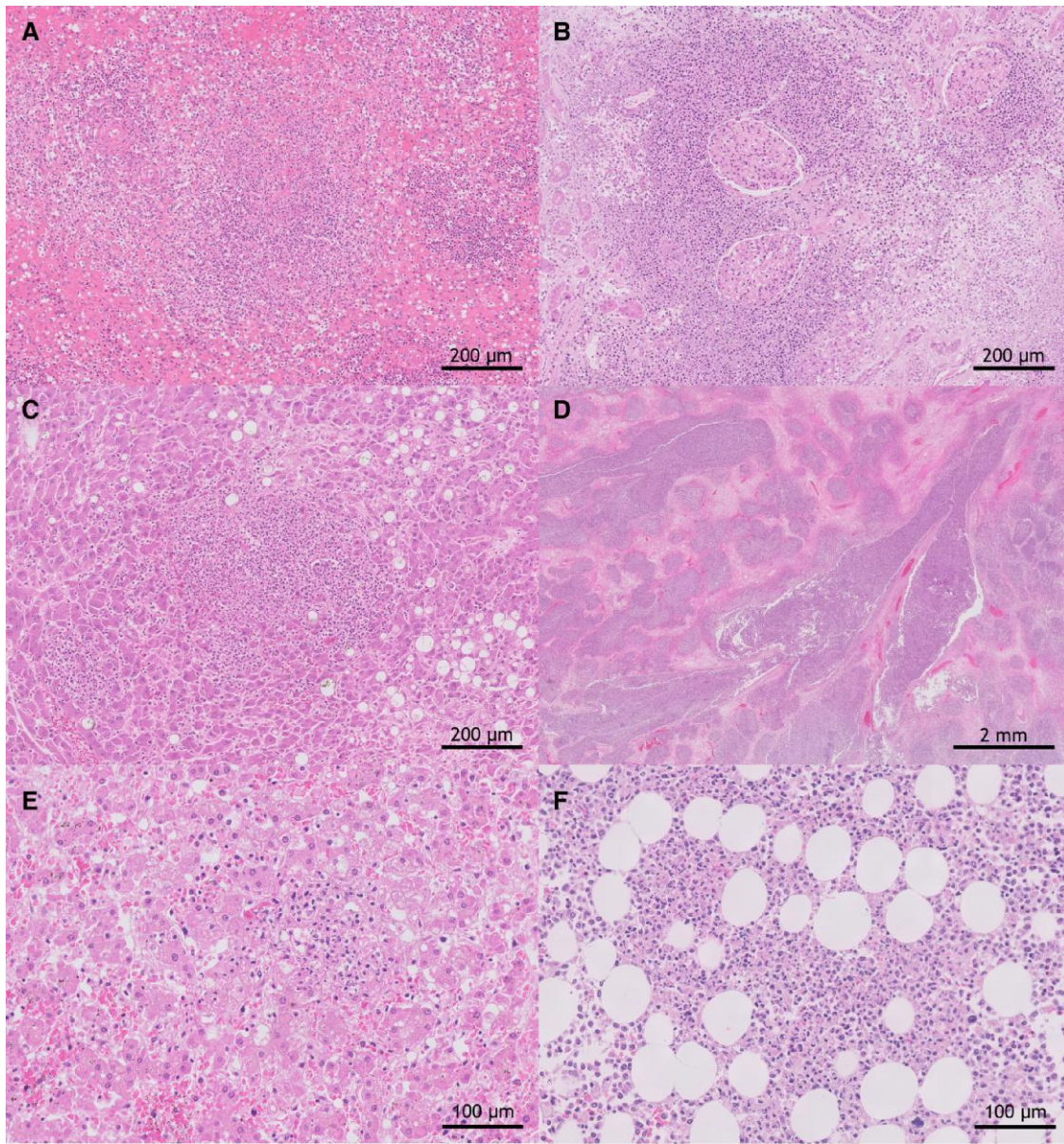


Figure 3. Histopathology of extrapulmonary manifestations of fatal melioidosis. Images all show hematoxylin and eosin staining. (A) Splenic abscess in C1. (B) Renal abscess in C2. (C) Liver abscess in C3 with a background of macrovesicular steatosis. (D) Extensive suppurative prostatitis in C4. (E) Adrenal abscess in C5. (F) Osteomyelitis of bone marrow with abscess and necrosis in C5.

involvement of the mediastinal lymph node and the resected stomach. Inhalation and ingestion have previously both been implicated in contracting melioidosis in a case-control study [29]. Mediastinal melioidosis was present in one of our biopsy cases (C10). The Darwin Prospective Melioidosis Study reported mediastinal lymphadenopathy/mass to be present in 99 cases, and it has been proposed that this form of disease is due to inhalation of *B pseudomallei* [9]. This is further illustrated by a case report from Australia that established a genomic match between the clinical isolate of a case with pulmonary and mediastinal involvement and isolates collected by environmental air

sampling [30]. Severe weather events in Australia and Singapore have been shown to increase the likelihood of pulmonary presentations of melioidosis, supporting a shift from percutaneous to inhalation as portal of entry [31, 32]. We were further able to establish the presence of *B pseudomallei* in tissue of a resected stomach by using the CPS staining (C9). This supports ingestion as another possible route of infection, although involvement of the stomach is not frequently encountered, as evidenced by the reporting of only 10 cases with a gastro/paraintestinal mass in the Darwin Prospective Melioidosis Study [9]. It is interesting to note that *B pseudomallei* has been detected in

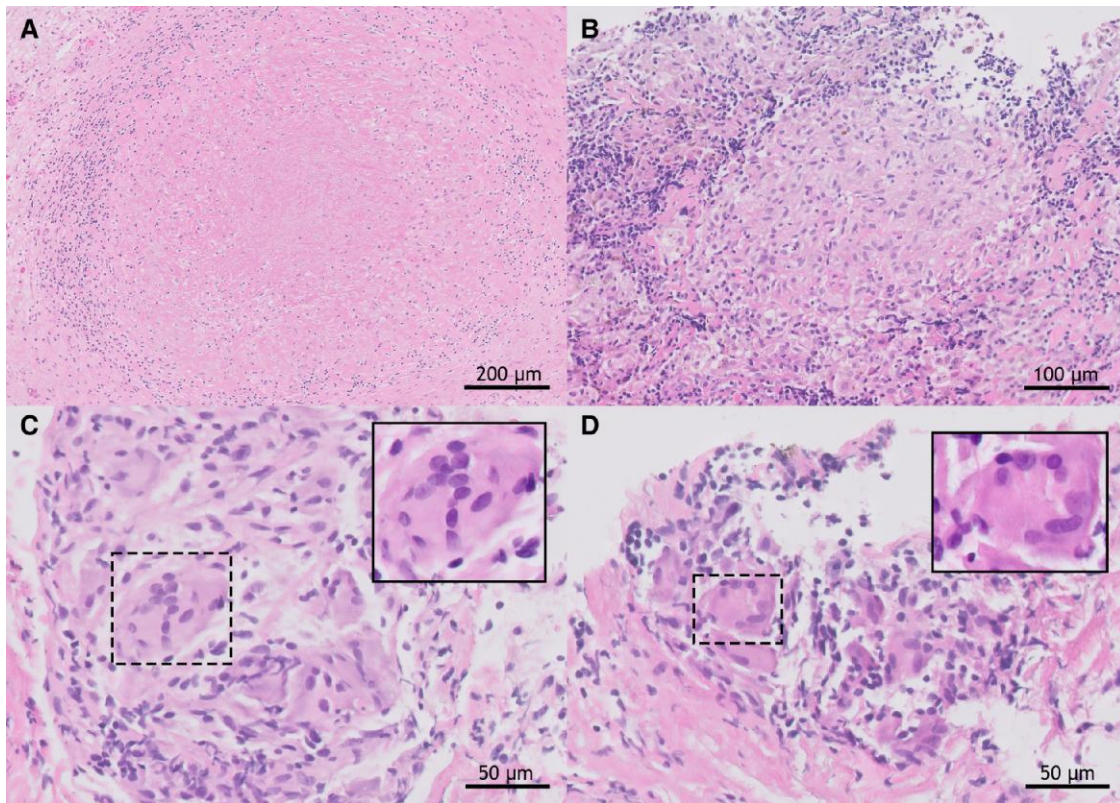


Figure 4. Histopathology of biopsy specimens. Images all show hematoxylin and eosin staining. (A) Granuloma and necrosis in tissue of a resected stomach in C9 with a chronic duration of symptoms. (B) Mediastinal lymph node of C10 showing granulomatous inflammation. (C and D) Examples of multinucleated giant cells in mediastinal lymph node tissue of the same case C10.

gastric biopsies of cases without symptoms pointing to melioidosis in Malaysia, but similar efforts in Thailand were unsuccessful [33, 34]. Nevertheless, the study in Thailand did show the presence of *B pseudomallei* in stool and rectal swabs of melioidosis cases, although the significance of these findings remains uncertain because it could simply reflect patients with melioidosis pneumonia swallowing sputum containing *B pseudomallei* [34]. Gastrointestinal presence of *B pseudomallei* combined with an absence of symptoms is in line with an experimental mouse study that showed *B pseudomallei* to be able to colonize the gastrointestinal tract, with a preference for the stomach, after oral or intranasal inoculation, suggesting this site to serve as a reservoir for disease [35].

Strengths and Limitations

The strengths of our study include the rigorous approach of only selecting tissue sections of cases with site-specific positive cultures for *B pseudomallei* and/or positive CPS staining. This assured that we only included melioidosis-specific histopathological features in our graphical overview. All organs commonly associated with melioidosis have been included (eg, lung, liver, spleen, prostate). In addition, more rare disease

manifestations have been included, such as stomach, resulting in an extensive melioidosis “atlas”.

A limitation of our study is the limited number of cases and absence of organs uncommonly associated with melioidosis, such as involvement of the central nervous system, the pathology of which has been described previously [36]. Availability of tissue was another limiting factor because some of our autopsy cases had more affected organs/tissue than were available for analysis, and we excluded negatively CPS-stained tissue (ie, autopsy cases only), which occasionally displayed features that could have been compatible with melioidosis. For example, the latter included an autopsy case with culture-confirmed kidney and mediastinal involvement in which both were negative on CPS staining. Explanations for the negative CPS staining include sampling errors or septicemia that prompted positive cultures. As a result, there is a high probability that selection bias and sampling bias have occurred. Due to the limited number of cases, we provided a descriptive graphical overview without formal statistical analysis. Histopathological data remain scarce within the current body of literature on melioidosis. Our case series aims to add a systematic graphical resource, displaying characteristics of acute and chronic stages of human melioidosis in different organs.

Implications for Practice

Clinical, radiological, and histopathological features of melioidosis may be misrecognized as tuberculosis or other infections, thus hindering a correct diagnosis [16, 37–39]. Health professionals need to consider melioidosis when confronted with disseminated disease alongside abscess formation and/or a necrotizing or granulomatous inflammation, and regardless of the presence of multinucleated giant cells. Thus, histological clues include (confluent) necrosis, (extensive) abscess formation, granulomata, and multinucleated giant cells. Because these features overlap with tuberculosis, pathogen-specific immunohistochemistry can be of adjunctive diagnostic utility. Indeed, it was immunohistochemistry upon autopsy that partly led to the organ-specific diagnosis of melioidosis in one of the cases of the multistate outbreak in the United States [8]. The CPS staining can be especially useful for stored tissue from diagnostic dilemma cases with possible melioidosis but in instances in which previous cultures were negative or not available.

CONCLUSIONS

In conclusion, we found fatal melioidosis to be a necrotizing and suppurative inflammation with presence of large numbers of neutrophils in the acute stages of disease. The CPS staining proved beneficial in establishing support of a histopathological diagnosis. Our graphical overview can be used as an aid by infectious diseases specialists, microbiologists, and pathologists.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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Potential conflicts of interest. All authors: No reported conflicts of interest.

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