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Appendicolith classification: physical and chemical properties of appendicoliths in patients with CT diagnosed acute appendicitis – a prospective cohort study

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ABSTRACT

Objective Appendicoliths are associated with a more complicated course of acute appendicitis and failure of non-operative treatment. We aimed to update the appendicolith classification originally described in 1966 and to assess the association of appendicolith characteristics with appendicitis severity.

Design This prospective predefined MAPPAC-trial (ClinicalTrials.gov NCT03257423) substudy included patients with CT diagnosed appendicitis presenting with an appendicolith. CT visible appendicoliths were harvested at surgery, measured and characterised by morphological examination complemented with micro-CT and micro-X-ray fluorescence spectroscopy. Patients were categorised into two groups: appendicolith appendicitis without other complications and appendicolith appendicitis with complications (appendiceal gangrene, perforation and/or abscess). The association of appendicolith classification and characteristics with appendicitis severity was evaluated.

Results Of 78 patients with a CT appendicolith, 41 appendicoliths were collected and classified based on the degree of hardness into three classes. The hardest appendicoliths (class 3) were less common (19.5%) presenting with a stone-hard outer layer and concentrically layered inner structure around a core. The layered inner structure was also observed in class 2 appendicoliths, but was absent in soft, class 1 appendicoliths. Appendicolith hardness or measures (maximum length, diameter and weight) were not associated with appendicitis severity. The spatial distribution of the main inorganic elements of calcium and phosphorus varied within most appendicoliths.

Conclusion This updated classification confirms categorisation of CT visible appendicoliths into three classes based on their physical and chemical characteristics. The data on clinical and aetiopathological characteristics of appendicoliths is scarce and using this systematic classification would add to this understanding.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Already in 1966, appendiceal concretions were classified in the Gut based on their physical characteristics as a faecal pellet, calcified faecolith or calculus. This classification is due for an update.

⇒ The finding of an appendicolith on CT in adult patients with acute appendicitis is associated with a more complicated course of acute appendicitis and failure of antibiotic treatment of appendicitis.

⇒ The presence of an appendicolith does not always lead to perforation, but the variability of appendicolith characteristics is poorly understood.

WHAT THIS STUDY ADDS

⇒ All appendicoliths, including the soft appendicoliths (class 1), congruent to faecal pellets in the original 1966 classification, are visible by CT.

⇒ Harder appendicoliths (class 2 and 3) are characterised by a concentrically layered inner structure built around the distinguishable core.

⇒ The main inorganic elements in all classes are calcium and phosphorus, but the proportion and distribution of elements vary.

HOW MIGHT THIS STUDY AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The presence of an appendicolith in patients with acute appendicitis is associated with a higher risk of perforation and failure of non-operative treatment. This study on patients with acute appendicitis highlights that the whole spectrum of appendicoliths from soft to stony are CT visible with potential clinical relevance in the treatment options for uncomplicated acute appendicitis.

⇒ This updated classification using various modalities allows us to better understand the appendicolith characteristics and structure adding to the understanding of the clinical classification of appendicoliths and aetiopathogenesis of acute appendicitis.

INTRODUCTION

Acute appendicitis was previously thought to always lead to perforation. However, we now know that complicated and uncomplicated acute appendicitis are two different diseases epidemiologically and clinically suggesting also different pathophysiology.[1 2] CT can quite reliably be used to differentiate between complicated and uncomplicated appendicitis.[3] This is of clinical importance as complicated appendicitis most often requires emergency appendicectomy, non-operative treatment with antibiotics is feasible and even symptomatic treatment may be sufficient in patients with uncomplicated acute appendicitis.[2 4–8] According to increasing evidence, the presence of an appendicolith is associated with a more complicated course of the disease[3 9 10] and failure of non-operative treatment for uncomplicated acute appendicitis.[4 11] Appendicolith is the most common finding of potentially complicated acute appendicitis with a prevalence of appendicolith as high as 39% in patients with CT-confirmed appendicitis[12] underlining the importance of bridging the knowledge gap of appendicolith pathogenesis and characteristics associated with appendicitis severity.

In 1966, Forbes and Lloyd-Davies described in detail the different forms of faecal concretions found in the appendix and categorised them into three classes according to physical characteristics and degree of hardness. The classes were defined as (1) faecal pellet, (2) calcified faecolith and (3) calculus, with class 1 being the most common and class 3 the rarest.[13] Already in 1921, appendiceal concretions were reported to contain an organic fat-soluble part composed of cholesterol, coprosterol and soaps, and an inorganic part containing mainly calcium.[14] However, since then the physical and chemical composition of appendicoliths has not been elaborated on. A recent study of five appendicoliths from paediatric patients characterised calcium and phosphorus as the major inorganic elements and a variety of fatty acids and proteins.[15]

The aim of this prospective study was to systematically assess CT visible appendicoliths both clinically and using a wide translational approach in conjunction with thorough clinical data in patients presenting with both acute appendicitis and an appendicolith. In addition to updating the 1966 morphological classification and its association with appendicitis severity, this study aims to assess the internal structure of appendicoliths with micro-CT (micro-CT) and investigate the main elemental composition with micro-X-ray fluorescence spectroscopy (micro-XRF).

MATERIALS AND METHODS

Study population and sample collection

This prospective observational cohort study was part of the MAPPAC (Microbiology Appendicitis Acuta) trial (ClinicalTrials.org, NCT03257423) with this substudy including patients with CT-confirmed complicated acute appendicitis. Patients were recruited at Turku University Hospital in Finland, between 11 April 2017 and 29 March 2019. The study was performed in accordance with the Declaration of Helsinki and all patients gave written informed consent to participate in the study. The study design and protocol have been previously reported in detail.[16] Briefly, the MAPPAC trial recruited patients with uncomplicated appendicitis participating in concurrent randomised controlled trialsAppendicitis Acuta (APPAC) II[17] and APPAC III[7] evaluating non-operative treatment of uncomplicated acute appendicitis, as well as patients presenting with uncomplicated and complicated appendicitis not participating in these trials, and treated with an emergency appendicectomy. Appendicoliths were defined as highly attenuated round or oval concretions ≥3 mm visible on CT. Complicated appendicitis was defined as the presence of an appendicolith, perforation (transmural inflammation and visible perforation), abscess (transmural inflammation with a phlegmon, possible visible perforation), gangrene (transmural inflammation and necrosis of the appendiceal wall) or the combination of these. The differential diagnosis of complicated acute appendicitis using these criteria was confirmed by assessing CT and surgical findings as well as the histopathological examination of the appendix.[7 17] Appendicoliths were collected immediately after appendicectomy into a sterile container and initially, they were stored at +4°C for up to 48 hours and then transferred to −80°C until further processing. Appendiceal samples were also collected from these patients to study the microbiology of acute appendicitis. The appendiceal tissue samples were not a part of this substudy and are not reported here.

Appendicolith characterisation and classification

All appendicoliths were classified into three categories based on the degree of hardness of the outer surface. This classification adapts the 1966 classification[13] with the major distinction that only CT visible appendicoliths were included in the grading and the classification was done solely based on the hardness of the outer surface. Similarly, to the original classification, class 1 appendicoliths were defined by the ability to be squeezed by tweezers in contrast to class 2 and 3 appendicoliths. Unlike others, class 3 appendicoliths made a distinct clinking sound when dropped on the metal surface. The classification was performed in the laboratory by one investigator (SV) to ensure consistency in the subjective evaluation. In cases of uncertain classification, the appendicolith was assessed and categorised based on joint evaluation by two investigators and consensus was reached through discussion. The classification was aimed to be performed prior to freezing. In nine appendicoliths this was not possible due to the acute care surgery nature of this prospective cohort study and emergency surgery schedules and these appendicoliths were classified only after melting. Appendicolith hardness for the already classified appendicoliths was observed also after melting confirming that the freezing did not affect the classification.

Appendicoliths were weighed and measured before analysis right after removal from the −80 freezer to avoid desiccation. If an appendix contained several appendicoliths, the largest one was selected for characterisation and analysis. Appendicoliths were morphologically characterised, measured and both surface and cross-sectional photographs were taken using Canon EOS 60D digital camera and a Canon EF-S 60 mm macro lens.

For elemental analysis, 15 representative appendicolith samples (5 from each class) were selected based on the sample collection order. Selection was done after excluding samples from patients that had a body mass index (BMI) over 30 kg/m^2 to mitigate potential bias related to the CT imaging as these patients underwent standard CT imaging and patients with a BMI<30 kg/m^2 underwent low-dose CT according to the APPAC II and III study protocols. We also excluded samples that were not classified before freezing to minimise bias in the classification. Patient sex was also considered in the representative sample selection by including both males and females. Due to limited resources, a further selection of six representative appendicoliths was done (two from each of the three classes) for micro-CT measurements.

Initial CT imaging

Contrast-enhanced CT images in the venous phase were obtained from patients with suspected acute appendicitis. The initial CT images for all patients taken at the emergency department were evaluated by two experienced specialists in abdominal radiology in retrospect to assess the radiographic characteristics of the appendicoliths, for example, appendiceal diameter. The radiographic hardness of the appendicoliths was measured by analysing the radiographic density with Hounsfield units (HU). The regions of interest were measured with an oval shape to cover 80–90% of the appendicoliths surface area at the largest diameter using Philips PACS, Philips Medical Systems, V.12.

Micro-CT

The X-ray microtomography (XMT), that is, micro-CT measurements were performed using a high-resolution XMT scanner (Phoenix Nanotom 180 NF, GE Measurement and Control Solutions GmbH, Germany).[18] Imaging was performed with a divergent cone beam configuration using a source voltage of 60 kV and a current of 150 µA. The imaging parameters were selected to provide a voxel size of 15 µm. A total of 720 transmission radiographs were taken from each sample. The tomographic reconstruction of the transmission images was done with Datoslx software supplied by the manufacturer. The image processing of the reconstructions was performed with Dragonfly software V.2020.2 and V.2022.2 (Object Research Systems, Canada; software available at http://www.theobjects.com/dragonfly).

Micro-X-ray fluorescence spectroscopy

To obtain a flat cross-sectional surface for micro-XRF analysis, the appendicoliths were first dissected and the surface was trimmed with a microtome. For microtome trimming, half of the appendicolith was embedded in paraffin. Micro-XRF analysis was performed with a Bruker Tornado M4 (Bruker Nano GmbH, Germany). X-rays were generated using an Rh tube operating at 50 kV with a source current of 600 µA. Elemental maps were acquired using a spot size of 20 µm obtained with polycapillary optics, step size of 50 µm and a dwell time of 20 ms. The instrument was calibrated with a manufacturer-provided reference sample block. Quantitative analysis of elements from the micro-XRF maps was also calculated.

Organic elemental analysis

The carbon, hydrogen and nitrogen (CHN) content of the appendicoliths were analysed with Flash 2000 CHNS organic elemental analyser (Thermo Fisher Scientific, USA). The instrument was calibrated using a sulfanilamide standard.

Statistical analysis

The association of the appendicolith hardness and appendicolith measures (maximum length, width and weight) to appendicitis severity was assessed by dividing patients into two subgroups; patients with an appendicolith appendicitis but no other signs of complications (appendicolith appendicitis) and patients with an appendicolith and other signs of complicated acute appendicitis (severe appendicolith appendicitis). Severe appendicolith appendicitis was defined as the presence of gangrene, perforation, abscess or the combination of these in addition to the appendicolith. Appendicolith classification on the degree of hardness (class 1–3) as a categorical variable was expressed as frequency with percentages, and Fisher's test was used for the analysis. Continuous variable radiographic density in HU units with non-normal distribution was presented as median (range) and the correlation between the three classes and HU units was evaluated with Spearman's correlation test. Kruskal-Wallis test was used to compare the diameter and HU units from collected and analysed to uncollected appendicolith samples. Continuous variables of maximum appendicolith length, width and weight were analysed using univariate logistic regression expressed as OR and 95% CI. A two-sided p value<0.05 was considered statistically significant. Statistical analyses were performed with SAS System for Windows, V.9.4 (SAS Institute, Cary, North Carolina, USA).

Patient and public involvement

Neither patients nor the public were involved in the design of this study. On recruitment, patients were informed of all aspects of the study when signing the informed consent, but the patients did not assess the burden of study participation themselves.

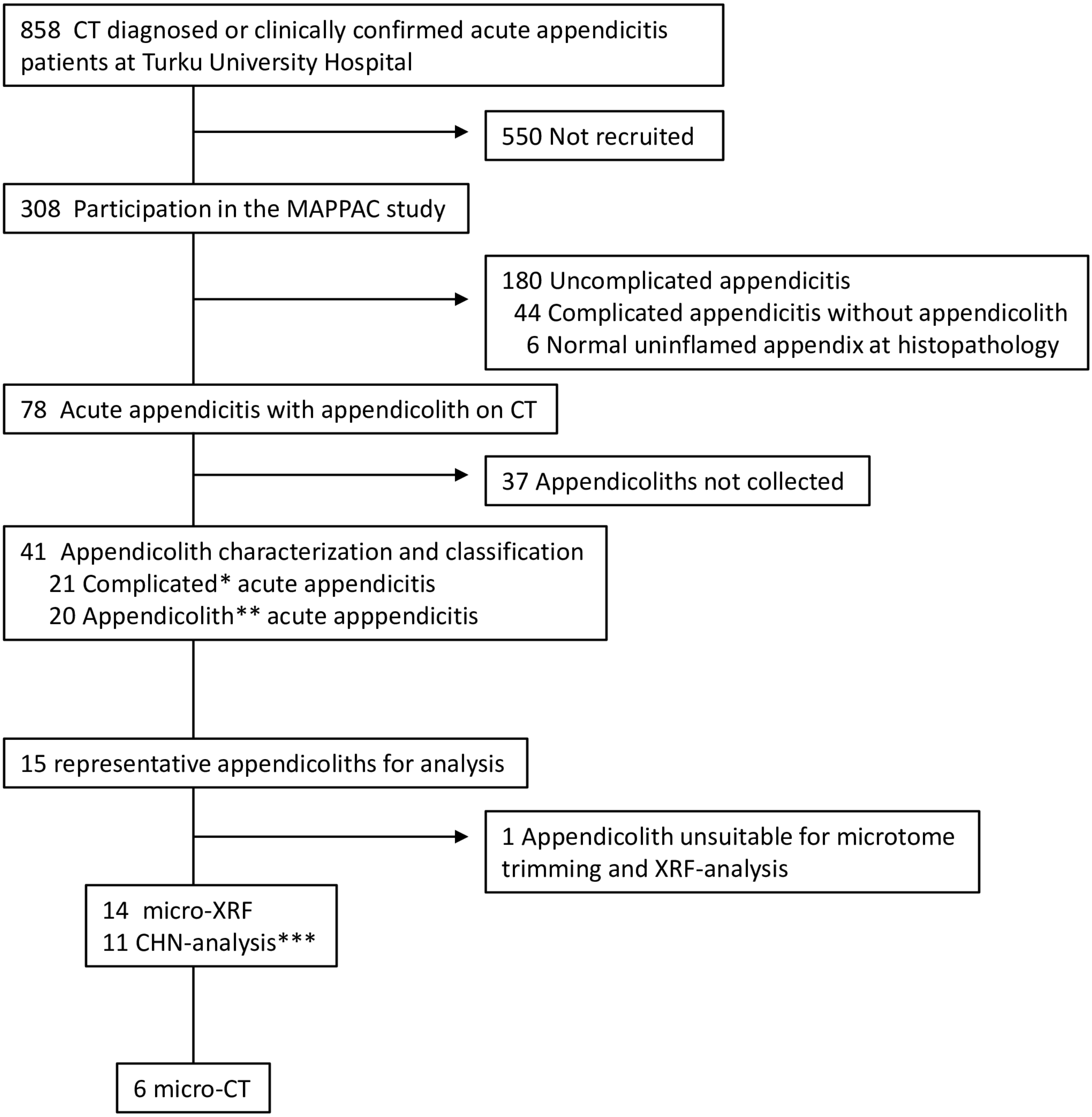

Figure 1 Flow chart of the study design. From 41 collected appendicoliths 15 representative appendicoliths were selected for elemental analysis including micro-X-ray fluorescence spectroscopy (micro-XRF) and carbon, hydrogen and nitrogen (CHN) analysis. *Another complication (gangrene, perforation and/or abscess) in addition to the appendicolith**The presence of an appendicolith with no other finding of complicated acute appendicitis***N3 Insufficient amount of appendicolith left for CHN-analysis.

RESULTS

Altogether 308 patients were enrolled in the MAPPAC study between 11 April 2017 and 29 March 2019. Out of the 122 patients with confirmed complicated acute appendicitis, 78 (63.9%) patients presented with an appendicolith on CT imaging and out of these, appendicolith(s) were available for collection from 41 (52.6 %) patients for characterisation and analysis. Patient flow and the performed appendicolith analyses are illustrated in figure 1. The clinical characteristics of the 41 patients are presented in table 1. Altogether 21 patients (51.2%) with a CT visible and harvested appendicolith were classified to have a more severe form of complicated appendicitis, that is, in addition to appendicolith, another complication was present (gangrene, perforation and/or an abscess). In 20 (48.8%) patients, there was only the appendicolith without any other findings of complicated acute appendicitis. There were 20 patients (48.8%) presenting with more than one appendicolith with a median of 2 appendicoliths (range 2–6).

Table 1 Clinical characteristics of patients presenting with both an appendicolith and acute appendicitis

Characteristic	N=41
Age (years), mean (SD)	44.1 (14.1)
Sex, n (%)	
Male	23 (56.1)
Female	18 (43.9)
BMI (kg/m^2), mean (SD)	27.3 (4.8)
Appendicitis severity n (%)	
Appendicolith without any other complications	20 (48.8)
Appendicolith with gangrene/perforation and/or abscess	21 (51.2)

BMI, body mass index.

Appendicolith classification and characterisation

A total of 41 appendicoliths were included in the final characterisation and classification (table 2); class 1 (17/41, 41.5%), class 2 (16/41, 39.0%) and class 3 (8/41, 19.5%). Class 1 appendicoliths were defined as formed but soft concretions, class 2 appendicoliths had firm to hard surfaces and class 3 appendicoliths were stony hard on the outer surface. Morphological features of representative appendicoliths from each class are presented in figure 2A. Class 1 appendicoliths were the smallest in all measurements. Class 2 appendicoliths had the longest maximum measure, while class 3 appendicoliths were the heaviest. In all classes, the majority of appendicoliths were round or oval.

In 23.8% of the patients (n=5/21) with an appendicolith and some other finding of complicated appendicitis presented with a class 3 appendicolith; class 3 appendicoliths were present in 15.0% of the patients with only an appendicolith appendicitis (p=0.85). Similarly, there was no statistically significant association between appendicitis severity and appendicolith maximum length (OR 1.03, 95% CI 0.91 to 1.17, p=0.60), diameter (OR 1.05, 95% CI 0.84 to 1.30, p=0.68) or weight (OR 1.00, 95% CI 0.99 to 1.00, p=0.45).

The appendicoliths in class 1 had a median radiographic density of 174.0 HU (37–337 HU), class 2 201.0 HU (138–371 HU) and class 3 had the highest radiographic density and most closely resembled bone with a mean attenuation of 486.0 HU (263–730 HU). The three-class classification of appendicoliths based on appendicolith hardness correlated with the attenuation in the CT images (online supplemental figure 1, Spearman coefficient 0.74, p<0.001). For comparison, HU values and diameters were measured also for patients whose appendicoliths were not collected as samples for analysis. The median attenuation for the collected appendicoliths was 187.0 HU (37–730 HU) and for the uncollected appendicoliths 222.0 HU (75–1293 HU), respectively (p=0.52). Collected appendicoliths were statistically significantly larger (longest maximum measure) than uncollected appendicoliths (median 11.0 mm (3.0–20.0 mm) vs 8.0 mm (3.0–30.0 mm), p=0.0026).

In addition to the morphological characterisation, the three-dimensional structure of six representative (two from each class) appendicoliths was determined with micro-CT. Both the visible structure on the cross-sectional surface as well as the X-ray attenuation pattern in the micro-CT slice (figure 2A,B) demonstrated that class 2 and 3 appendicoliths have a concentrically layered structure formed around a core. In most (71%) appendicoliths in class 1 (12/17), both a distinct core and the layered structure were absent. With micro-CT imaging, the stony hard outer surface in class 3 appendicoliths showed high X-ray attenuation appearing continuously bright, whereas soft class 1 appendicoliths did not have a separate outer layer (figure 2B and online supplemental video). The core in most appendicoliths was softer also indicated by lower attenuation that appears darker in the cross-sectional micro-CT image.

Elemental distribution of appendicoliths

The elemental distribution on the appendicolith dissection surface was determined from 14 representative

Table 2 Appendicolith classification and physical characteristics. Appendicolith weight, maximum length and diameter are expressed as mean (range)

Appendicolith class	Description	Occurrence n/41 (%)	Weight (mg)	Maximum length (mm)	Maximum diameter (mm)
Class 1	Clear form, but soft faecal-like structure. Cross-sectionally amorphous. No visible core.	17 (41.5)	243 (11–816)	9.9 (4–20)	6.3 (2–9)
Class 2	Firm to hard surface. Cross-sectionally concentrically layered structure. Visible core.	16 (39.0)	589 (19–1600)	12.6 (4–22)	8.0 (3–13)
Class 3	Stony hard outer surface. Cross-sectionally concentrically layered structure. Visible core.	8 (19.5)	646 (15–2985)	10.9 (6–19)	7.2 (3–14)

Maximum diameter of the appendicolith is the vertical measure of the maximum length.

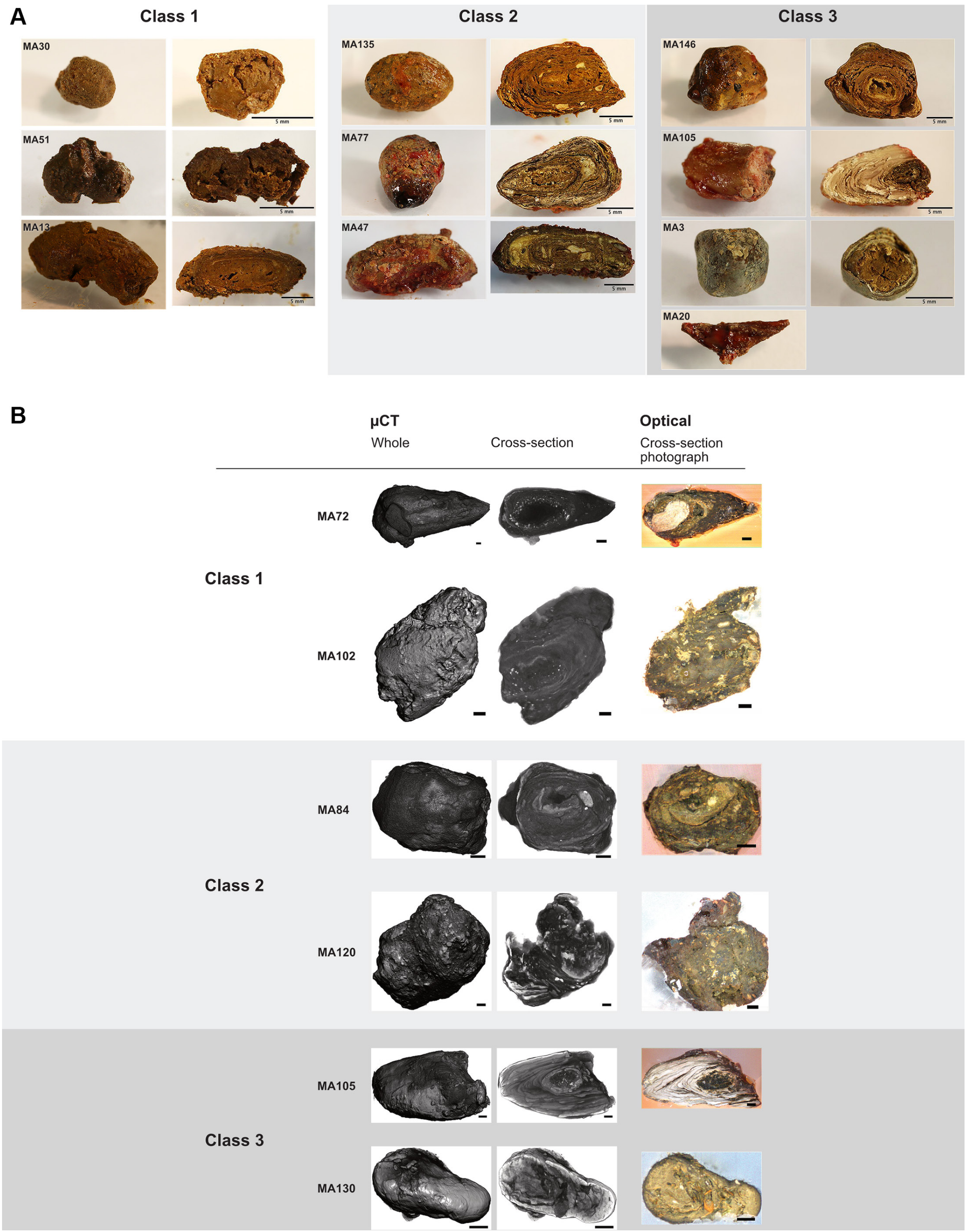

Figure 2 Appendicolith characteristics and structure. (A) Photographs of representative appendicoliths from the outer surface and cross-section classified by the degree of hardness (1–3). Class 1 appendicoliths appeared as formed but relatively soft faecal concretions. Class 2 appendicoliths showed layered structure without a stony hard surface layer. Class 3 appendicoliths had an extremely hard outer surface and layered inner structure. In all classes appendicoliths were mostly brown in colour, but in classes 2 and 3 yellowish layers or patches were visible in the cross-section and in class 3, there were individual appendicoliths that were almost white with dark core. A divergent appendicolith in class 3 (20) was triangular with sharp edges, possibly an ingested bone fragment. The scale bars are 5 mm. (B) Appendicolith structure classified by the degree of hardness in the six representative appendicoliths. The micro-CT surface renderings of appendicoliths show that appendicoliths have a diverse surface structure. The micro-CT slices through the middle of the stones reveal a distinguishable core and a layered structure in the hardest appendicoliths (class 2 and 3), which is also visible in the adjacent photograph of the paraffin-embedded cross-section. Appendicolith 105 most likely represented a broken-off half from a larger appendicolith, shown in 2A. The scale bars in all images are 1 mm.

appendicoliths. In all appendicoliths, the main elemental components were calcium and phosphorus (figure 3 and online supplemental table 1). The mean weight percentage of calcium and phosphorus increased along with the appendicolith hardness. The calcium-to-phosphorus atomic ratio varied between appendicoliths from 1.9 to 4.3. Titanium, iron, manganese and copper were found in trace amounts from all appendicoliths (online supplemental table 2).

The spatial distributions of calcium, phosphorus and potassium showed dissimilarities between different areas within most appendicoliths. The elemental compositions of core and shell areas were examined in more detail from those appendicoliths that showed a clearly visible core (online supplemental figure 2 and table 3). The core elemental composition of these appendicoliths was distinguishable from the shell area and comprised of varying elemental components.

Elemental analysis of carbon, nitrogen and hydrogen was performed to estimate the organic proportion of 11 representative appendicoliths (online supplemental table 4 for CHN-analysis.) The estimated average percentages by weight of organic proportion in class 1 was 44.4%, in class 2 33.8% and in class 3 36.4%.

DISCUSSION

This study assessing the characteristics and clinical correlation of CT visible appendicoliths confirmed that appendicoliths can be classified into the three classes originally proposed in the 1966 classification.[13] Additionally, we also demonstrated that the classification is associated with the internal structure of the appendicoliths. This updated classification in part adds to the previous knowledge of the association of appendicoliths with a more complicated course of appendicitis and failure of non-operative treatment.[4 11] To our knowledge, this is the first prospective clinical study to incorporate the characterisation of CT-positive appendicoliths from patients with complicated acute appendicitis and analyse the morphological, structural and elemental components in combination with thorough prospective clinical data. With this study, we were able to update and verify the original 1966 classification.[13] However, based on this study, no significant association between appendicitis severity and appendicolith characteristics (hardness, size, weight) could be demonstrated.

The distribution of appendicoliths between the three classes in this study was almost identical with the original classification differing only in the case of class 1 appendicoliths being CT visible compared with being described as radiotranslucent in the 1966 classification. Forbes and Lloyd-Davies suggested differentiating between a true stony hard calculus and a calcified faecolith or faecal pellet, with only the calculi requiring surgical treatment.[13] Since then, multiple studies have shown that a CT visible appendicolith is associated with increased risk for both complicated appendicitis, appendicectomy and failure of non-operative treatment.[3 4 9 11 19 20] To improve the standardisation of defining CT visibility of an appendicolith, Weitzner *et al* recently suggested the maximum HU value of an appendicolith to be 180 HU greater than HU values from appendiceal lumen or appendiceal wall.[21] In this study, we included all clinically relevant CT visible appendicoliths from appendicitis patients and classified them based on their degree of hardness and all CT visible appendicoliths were clinically significant in contrast to the suggested HU definition as class 1 appendicoliths that can have relatively low HU values. To unify the terminology, we suggest that in the case of appendicitis and appendiceal concretions, the terms faecalith or calculus would not be used and all appendicular concretions should be called appendicoliths no matter how calcified they are. Using standardised uniform classification and terminology in defining appendicoliths would enable and enhance comparison of different trials assessing patients with an appendicolith and acute appendicitis as appendicolith appendicitis is the most common form of complicated appendicitis.[12] From 41 characterised patients with appendicolith appendicitis, half of the patients (48.8%) showed no gangrene or perforation thus otherwise being uncomplicated and half of the patients (51.2%) showed more severe appendicitis with gangrene, perforation or an abscess in addition to the appendicolith. We estimated the appendicitis severity in different appendicolith classes, and while class 3 appendicoliths were slightly more common in the severe appendicitis group, no statistically significant connection between the appendicitis severity and the hardness of appendicoliths was found. In addition, appendicolith size (longest diameter, width and weight) was not associated with appendicitis severity. Only a few studies have assessed the effect of appendicolith characteristics on appendicitis severity. In contrast to our findings, Ishiyama *et al* showed appendicolith size of more than 5 mm, and the location of an appendicolith at the base of the appendix to be associated with gangrenous appendicitis.[10] In a paediatric study, appendicolith maximum diameter of 5 mm or more was a significant risk factor for perforated acute appendicitis.[22] A recent study suggested that patients with acute appendicitis presenting with an appendicolith under 5 mm and patients with a low C-reactive protein concentration might be treated conservatively, while an appendicolith diameter over 10 mm would present a high risk of appendiceal perforation, but no conclusions were drawn for the large group of patients with appendicolith diameter between 5 and 10 mm.[23]

In this study, with both visual characterisation and micro-CT imaging we observed that most appendicoliths in classes 2 and 3 have a layered structure built around the core of the appendicolith suggesting a gradual growth pattern implying that appendicoliths form layer by layer around the core and that appendicoliths actually are not simple faecal concretions, as was already stated in 1907.[24] Interestingly, the layered structure was absent in most class 1 appendicoliths and these softer appendicoliths

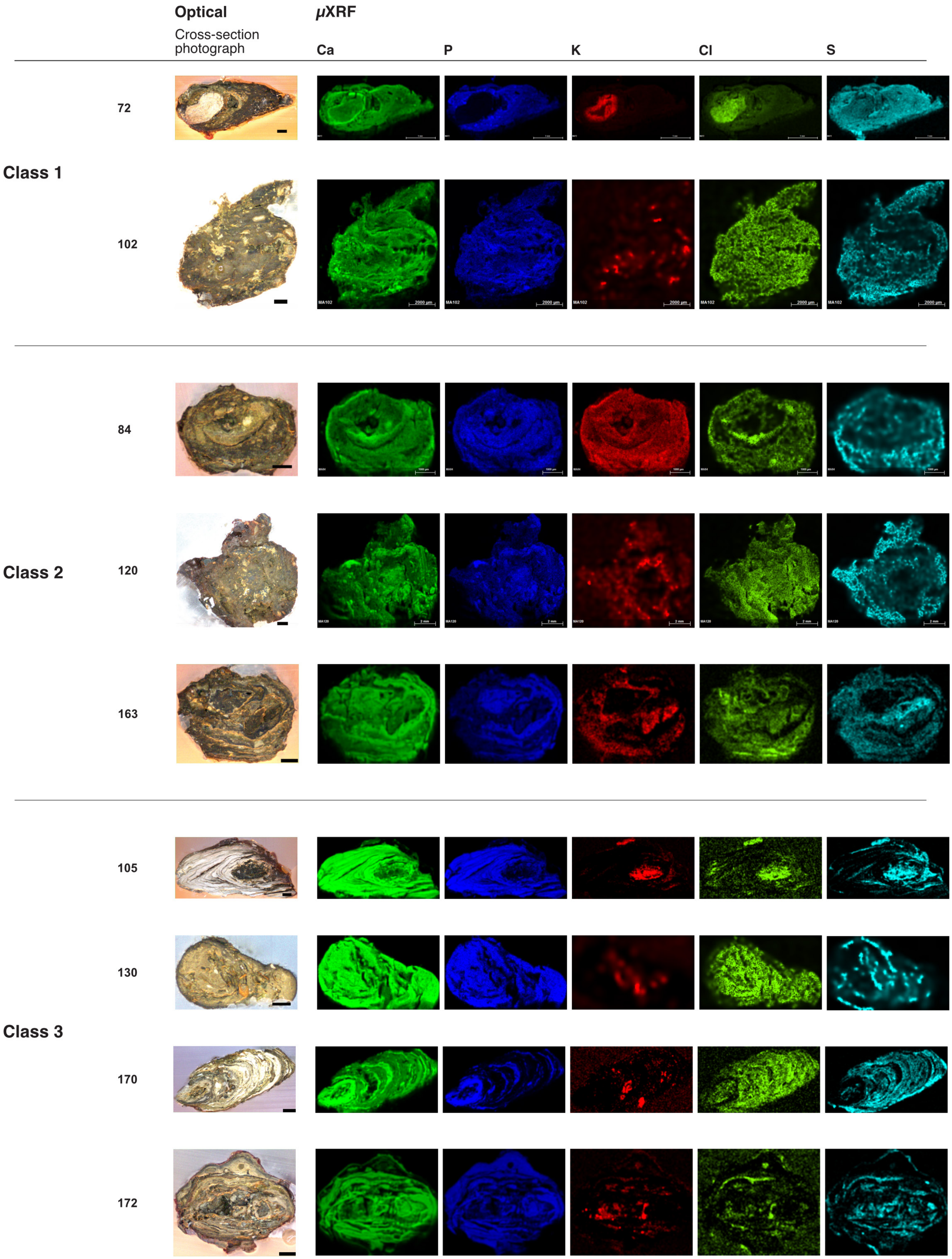

Figure 3 Photographs of the paraffin-embedded appendicolith cross-sections classified by hardness with the corresponding micro-XRF elemental maps of calcium (Ca), phosphorus (P), potassium (K), chlorine (Cl) and sulphur (S). In certain areas, calcium was clearly the main elemental component, such as in specimens 72 and 170 whereas in some areas calcium and phosphorus seemed to co-locate, for example, in 105 and 130. The scale bars in all photographs are 1 mm. XRF, X-ray fluorescence spectroscopy.

could perhaps represent younger appendicoliths as they were also smaller in weight and diameter compared with hardest class 3 appendicoliths. However, appendicoliths showed considerable heterogeneity both in external and in cross-sectional properties and structure, also within each class, thus suggesting that appendicolith formation could follow multiple different paths. With micro-XRF imaging we were able to show that in all classes, the main inorganic elements were calcium and phosphorus which is in line with previous studies.[14 15] However, the calcium percentage increased with the appendicolith hardness, and the calcium/phosphorus ratio varied between appendicoliths suggesting that calcium might be deposited as different compounds. Based on the elemental maps in combination with visual observation the core was often distinguishable from the outer layers of the appendicolith. Even though further research is needed to determine the organic compounds as well, the variation in the spatial distribution of elements inside appendicoliths and appendicolith core further supports the possibility of variable appendicolith formation pathways.

The exact pathogenesis of acute appendicitis and the role of appendicoliths is still unknown. According to one theory, appendicoliths are thought to commonly cause a mechanical obstruction of the appendiceal lumen resulting in infection and contributing to the development of acute appendicitis.[25] However, appendicoliths have also been found incidentally without signs of appendicitis on imaging.[26 27] Incidentally found appendicoliths do not increase the risk of developing appendicitis compared with the general population,[28] but several studies show that when appendicoliths present with acute appendicitis, the risk of perforation[29] and failure of non-operative treatment increases.[4 11 30] The longer-term follow-up of the large pragmatic Comparison of Outcomes of antibiotic Drugs and Appendectomy (CODA) trial found appendicectomy to be more common in patients with an appendicolith appendicitis, but this greater risk was attenuated over time with the highest risk for appendicectomy within 48 hours after diagnosis.[31] Thus, further knowledge regarding appendicoliths, their classification and their role in the pathogenesis of acute appendicitis is essential for a better understanding of the different forms of appendicitis and especially when considering non-operative treatment with antibiotics[5 8 17] or even symptomatic therapy.[7]

This study has several limitations. First, the inability to retrieve a significant number of the eligible appendicoliths constitutes a major limitation of the study leading to quite a small sample size. This was based on the acute care surgery setting of the study creating major challenges for patient enrolment. However, to our knowledge, this is the first prospective clinical study to incorporate CT imaging with morphological, structural and elemental components in combination with clinical data. In addition, there was no statistically significant difference in radiographic density of the collected and uncollected appendicoliths further supporting the generalisability of the classification. Second, only a subpopulation of appendicoliths were studied with micro-CT and micro-XRF due to limited resources. Even though appendicoliths were selected to be representative of the specific class, this might have caused selection bias especially due to the heterogeneity of the appendicoliths.

In conclusion

This morphological and structural analysis provides a necessary update of the 1966 appendicolith classification described before the era of modern CT in the diagnosis of acute appendicitis by confirming that CT visible appendicoliths can systematically be classified into three distinct classes. From a clinical standpoint, this appendicolith classification illustrates that even the softest stones were visible on CT with potential clinical relevance in the treatment options of uncomplicated acute appendicitis. Further research of the appendicolith characteristics in association with clinical course and aetiopathology using this systematic classification will markedly increase our understanding of acute appendicitis and the role of appendicoliths.

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Contributors PS, SS, EMu and AJH initiated the study and PS is the principal investigator. PS, SV, SS, EMä, EMu, JS, JG and AJH contributed to the implementation of the study, patient enrolment and data collection. SV, TS and EMä performed the laboratory analyses and did the analysis. SV and PS had full access to all the data in the study and verified the data. The manuscript was drafted by SV which was refined by PS, SS, EMä, EMu, TS, JG, JS and AJH. All authors approved the final version and PS had final responsibility for the decision to submit for publication and is the guarantor.

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Competing interests EMu is currently working as full-time Medical Advisor for Biocodex Nordics. PS reports receiving personal fees for lectures form Merck and Orion Pharma. AJH reports receiving personal fees for lectures from BioCodex, Merck and Pfizer. All other authors declare no competing interests.

Patient consent for publication Not applicable.

Ethics approval This study has been accepted by the Ethical Committee of the Hospital district of Southwest Finland (Turku University Hospital, approval number ATMK:142/1800/2016) and Finnish Medicines Agency (Fimea). The MAPPAC trial protocol is available in the online supplemental file 1. Participants gave informed consent to participate in the study before taking part.

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Data availability statement Data are available upon reasonable request. Data are available upon reasonable request. If interested in the raw data and de-identified patient data, please contact the corresponding author and data sharing will be evaluated based on the request.

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