

Crystals in Synovial Fluid: Diagnostic Methods and Clinical Relevance

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Abstract

Crystal analysis in synovial fluid (SF) is crucial for diagnosing crystal induced arthropathies which are an important cause of acute and chronic joint inflammation. Identification of crystals in synovial fluid remains a key diagnostic step in the evaluation of patients presenting with arthritis. The direct identification of crystals in synovial fluid remains the clinical reference standard for confirming gout and calcium pyrophosphate deposition disease (CPPD), while the recognition of less common particles such as basic calcium phosphate (BCP) and cholesterol crystals can also provide clinically relevant information. This review summarizes the literature from 2017 to 2026 on crystals in synovial fluid, with particular emphasis on crystal types, detection methods and their clinical relevance. It aims to provide a practical, clinician-oriented review of SF crystal analysis, highlighting crystal morphology, detection techniques, disease associations and clinical interpretation. Monosodium urate (MSU) and calcium pyrophosphate (CPP) crystals remain the most diagnostically important.

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Compensated polarized light microscopy remains the gold standard for crystal identification, but it is limited by observer dependence, low crystal burden, weak birefringence and artifacts. Newer techniques, especially Raman spectroscopy and other chemistry-based approaches, are emerging as promising adjuncts. Imaging methods such as ultrasound and dual-energy CT increasingly complement aspiration-based diagnosis but do not replace synovial fluid examination, particularly when infection is a concern. Recognition of synovial fluid crystals has important clinical implications, as crystal-induced arthritis may mimic or coexist with other joint diseases, including septic arthritis. Improved diagnostic techniques and continued clinician training may enhance the accuracy and clinical utility of synovial fluid analysis. SF crystal analysis is a practical, rapid, and reliable diagnostic tool. Familiarity with crystal morphology, detection methods, and clinical implications improves diagnostic accuracy and guides therapy.

Keywords: synovial fluid, synovial fluid analysis, gout, calcium pyrophosphate deposition disease, crystal arthropathy, polarized light microscopy

1. Introduction

Crystal-induced arthropathies are a broad group of inflammatory joint diseases caused by the deposition of crystals in the synovial fluid and surrounding periarticular tissues. The most common ones are gout, which results from MSU crystals, and CPPD, caused by CPP crystal deposition. In addition, BCP crystals, including hydroxyapatite, are increasingly recognized as playing a role in joint pathology, particularly in degenerative and periarticular conditions [1,2].

Synovial fluid plays an essential role in joint function by lubricating articular surfaces and supporting cartilage nutrition. It is a complex mixture derived from plasma ultrafiltrate and macromolecules produced by synovial cells, such as hyaluronan and glycoproteins, which are responsible for its characteristic viscosity [3,4,5]. Importantly, synovial fluid is not a passive effusion, but a dynamic intra-articular environment that is in constant interaction with cartilage and synovium. Because of this, its cellular and non-cellular components closely reflect the underlying processes occurring within the joint [3].

Because synovial fluid is so closely linked to intra-articular pathology, its analysis provides diagnostic information that cannot be fully replaced by imaging or serum tests. One of the most important applications of this is the identification of crystals. The presence of crystals in synovial fluid triggers inflammation through activation of innate immune pathways, leading to

both acute and chronic arthritis. In gout, MSU crystals activate the NLRP3 inflammasome in macrophages, resulting in caspase-1 activation and the release of interleukin-1 β , a key mediator of the inflammatory response [2,6,7]. Similarly, CPP crystals induce inflammatory changes within the synovium, giving rise to a spectrum of clinical presentations ranging from acute crystal arthritis to chronic degenerative joint disease [2]. The pathogenesis of crystal-induced inflammation in synovial fluid is shown in Figure 1.

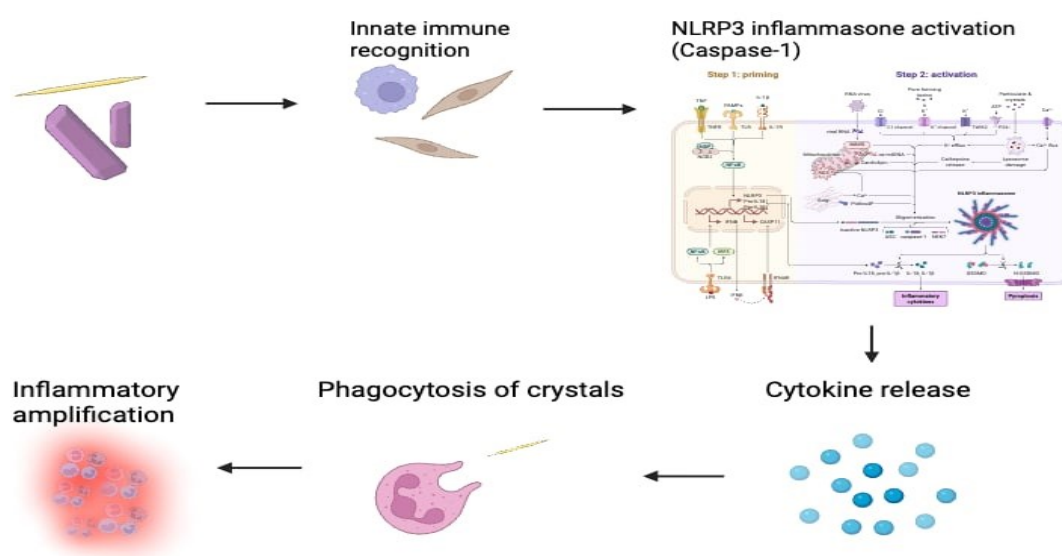


Figure 1. Pathogenesis of crystal-induced inflammation in synovial fluid

Accurate identification of crystals in synovial fluid is essential for diagnosing crystal-induced arthritis and guiding clinical management. Direct examination using compensated polarized light microscopy remains the gold standard, as it allows differentiation between MSU and CPP crystals based on their morphology and birefringence [8]. Current international recommendations state that the identification of MSU crystals in synovial fluid or tophus aspirates is sufficient to confirm gout [9]. Similarly, recent classification criteria for CPPD recognize the presence of CPP crystals in synovial fluid as a definitive diagnostic feature when supported by the clinical picture [10].

Despite its clear diagnostic value, synovial fluid crystal analysis has several limitations in routine clinical practice. The accuracy of crystal detection depends on technical factors such as sample preparation, microscope quality and, importantly, the experience of the observer. While identification of MSU crystals is generally reliable, studies show that CPP crystals and other crystal-like particles are more frequently misinterpreted under polarized microscopy [9]. In addition, some crystals, particularly BCP, are extremely small and non-birefringent, which makes them difficult to detect using conventional microscopy techniques [11].

Advances in diagnostic technologies have aimed to address these limitations. Imaging modalities such as ultrasound and dual-energy computed tomography (DECT) have improved the noninvasive detection of crystal deposits in joints and periarticular tissues [9,12]. In addition, emerging analytical approaches, including Raman spectroscopy and other spectroscopic techniques, have shown promising results in identifying crystal composition based on chemical signatures, offering the potential for more objective diagnostic tools in the future [5,11,13].

Another important aspect of synovial fluid crystal identification is its role in the differential diagnosis of acute arthritis. Crystal-induced arthritis can mimic septic arthritis and, importantly, may also coexist with infection in the same joint. Recent studies have shown that septic arthritis can occur even in patients with crystal-positive synovial fluid, which underscores the need for concurrent microbiological analysis whenever joint aspiration is performed [14,15].

In this context, this review provides a clinically oriented overview of crystals in synovial fluid, focusing on their morphology, detection methods and clinical relevance. It places particular emphasis on polarized light microscopy, while also addressing newer approaches such as spectroscopic techniques. The goal is to highlight key diagnostic challenges and practical points that can improve the interpretation of synovial fluid analysis in patients with suspected crystal-induced arthropathies. Despite technological advances, synovial fluid microscopy remains the only method that provides immediate, direct etiological diagnosis of crystal arthritis in routine clinical practice [8,9].

2. Search Strategy

For the purpose of this narrative review, we conducted a comprehensive search across relevant literature sources, including PubMed and Scopus, to identify articles on synovial fluid crystals. This review focuses primarily on literature from 2017 to 2026, while incorporating selected earlier landmark references for essential background context.

To ensure relevance, priority was given to clinically significant and high-quality evidence, including clinical studies, systematic reviews, meta-analyses, and international guidelines. This time frame was chosen to capture the most recent advances in the diagnosis and clinical interpretation of synovial fluid crystals.

We employed a structured set of keywords and search strings to target articles relevant to contemporary understanding of crystal arthropathies. The search terms included variations such as “synovial fluid crystals,” “crystal arthropathy,” “gout,” “calcium pyrophosphate deposition disease,”

“basic calcium phosphate crystals,” “cholesterol crystals,” “synovial fluid analysis,” “polarized microscopy,” “Raman spectroscopy,” and “crystal detection methods.” Reference lists of pertinent articles were also reviewed to identify additional studies that may have been missed in the initial search.

Exclusion criteria encompassed studies without clear clinical applicability. Articles were selected based on their relevance to crystal types, diagnostic approaches, and the clinical interpretation of synovial fluid findings. Selected textbook material, including Freemont and Denton’s *Atlas of Synovial Fluid Cytopathology*, was also incorporated to provide essential background context.

This narrative review was prepared with consideration of the SANRA (Scale for the Assessment of Narrative Review Articles) recommendations in order to improve methodological transparency, literature selection, and overall reporting quality.

3. Synovial fluid as a diagnostic medium

The diagnostic value of synovial fluid lies in the fact that it directly reflects the intra-articular environment. Beyond crystal identification, its analysis allows differentiation between inflammatory, non-inflammatory, and septic joint processes based on leukocyte count, differential, and biochemical features, making it a central tool in rheumatology practice [4,16]. Routine examination provides additional information on macroscopic appearance, viscosity, cellular content, microorganisms, and crystals. Recent literature continues to emphasize its role as one of the most useful laboratory investigations in joint disease, particularly in acute monoarthritis, where crystal arthritis and septic arthritis are the main diagnostic considerations [3,8,10,16].

Freemont and Denton’s pathology atlas remains an important reference for understanding why synovial fluid responds so sensitively to joint disease. It highlights that synovial fluid contains not only plasma-derived components, but also cells and macromolecules originating from the synovium and cartilage, and shows how pathological processes can alter cellular composition, fibrin content, and particulate material. This framework still fits well with current clinical practice, despite advances in diagnostic techniques.

In crystal arthropathies, synovial fluid analysis offers something uniquely valuable - direct confirmation of the causative crystal. This is particularly important because serum urate levels, imaging findings, and even typical clinical patterns can sometimes be misleading. The same applies to CPPD,

which has a wide range of clinical presentations and often overlaps with osteoarthritis and other inflammatory arthritides [9,17].

In addition to its role in crystal detection, synovial fluid examination may reflect underlying inflammatory mechanisms, as elevated levels of complement components in osteoarthritic joints suggest ongoing local activation of innate immunity and further highlight its value as a marker of joint pathology [18].

4. Crystals identified in synovial fluid and their appearance under polarized light microscopy

In routine clinical practice, crystal identification in synovial fluid relies primarily on compensated polarized light microscopy (CPLM), which allows differentiation of crystal types based on morphology and birefringence [2,15]. Among these, MSU and CPP crystals are the most clinically important, as their identification can establish the diagnosis of gout and CPPD in the appropriate clinical setting [9,17]. In contrast, BCP crystals are often not detected with standard microscopy, while cholesterol crystals and other less common particles are encountered more rarely but may still be relevant in specific clinical situations [2,5,11].

4.1. Monosodium urate crystals

MSU crystals are the hallmark of gout. They form when urate becomes supersaturated and precipitates in and around joints, and their identification in synovial fluid confirms the diagnosis of gout [8,9]. Morphologically, they are typically long, slender, needle-shaped crystals. Under compensated polarized light microscopy, they show strong negative birefringence: appearing yellow when aligned parallel to the slow axis of the compensator and blue when perpendicular [2,15].

MSU crystals are frequently observed intracellularly within neutrophils during acute gout attacks (Figure 2), a finding that strongly supports active crystal-induced inflammation [2,15]. Clinically, their identification is important not only in acute monoarthritis but also in chronic gout, intercritical gout, and atypical presentations, especially when imaging findings or serum urate levels do not provide diagnostic certainty [6,9]. This is particularly relevant because serum urate levels may be normal during acute gout flares, which further emphasizes the value of direct synovial fluid crystal identification [6].

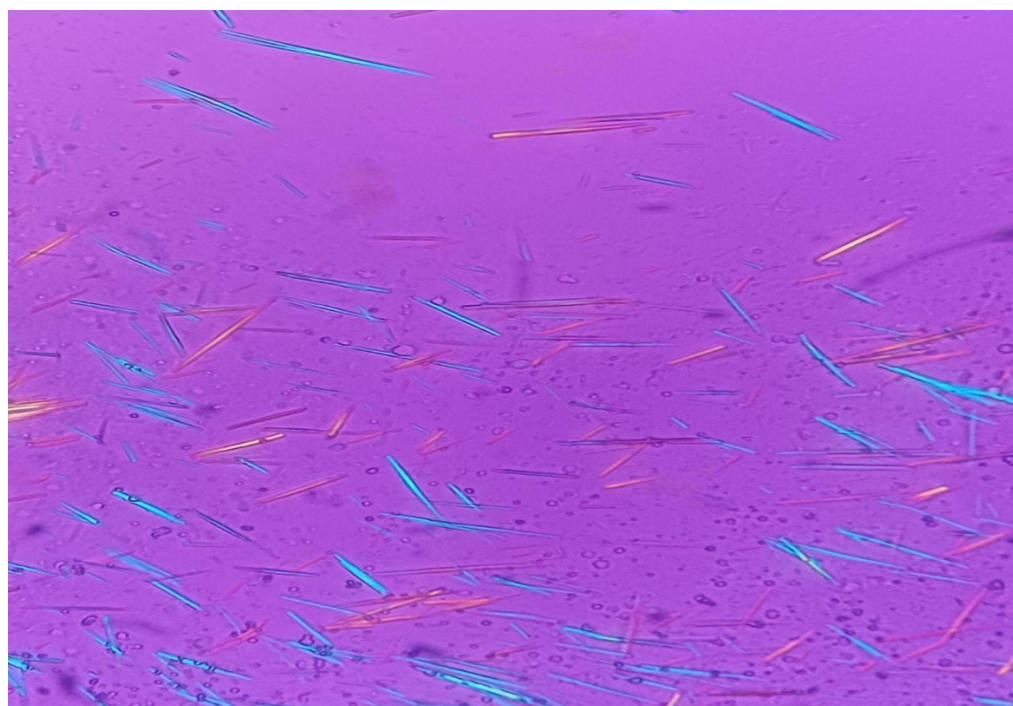


Figure 2. Monosodium Urate crystals in the tophus from a patient with gouty arthritis. Crystals are negatively birefringent and needle shaped, observed under polarized light microscopy, x63.

4.2 Calcium pyrophosphate crystals

CPP crystals are responsible for CPPD, a clinically heterogeneous condition that can present as acute CPP crystal arthritis, chronic inflammatory arthritis, osteoarthritis with CPPD, crowned dens syndrome, or asymptomatic chondrocalcinosis [9,14,17]. Under compensated polarized light microscopy, they are typically rhomboid, rod-shaped, or short rectangular and show weak positive birefringence, appearing blue when aligned parallel to the slow axis of the compensator and yellow when perpendicular [2,11,15]. Compared to MSU crystals, CPP crystals are more difficult to identify, as their birefringence is often faint and their morphology more variable, making them a common source of diagnostic difficulty in synovial fluid microscopy [2,11,15].

The diagnostic importance of CPP crystals has been reinforced by the 2023 ACR/EULAR classification criteria, which recognize crystal detection in synovial fluid as a core feature of symptomatic CPPD [4] (Figure 3 and 4). Recent literature also highlights the strong association of CPPD with aging, cartilage degeneration, and osteoarthritis, while noting that it can mimic seronegative rheumatoid arthritis and other inflammatory joint diseases [12,14,17,19,20]. CPP crystal formation is thought to result from alterations in cartilage metabolism, including increased extracellular pyrophosphate

production and reduced crystal clearance, both of which promote deposition in aging cartilage [19].

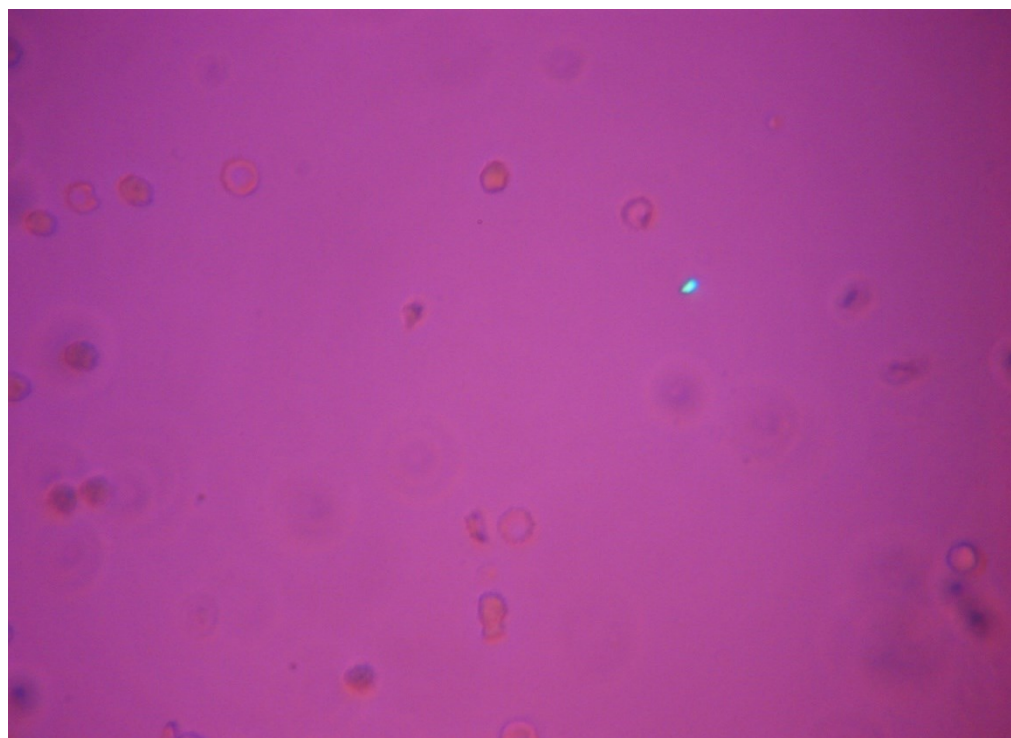


Figure 3. CPP crystals in the synovial fluid from a patient with acute CPP crystal arthritis. Crystals are positively birefringent and rhomboid shaped, observed under polarized light microscopy, x63.

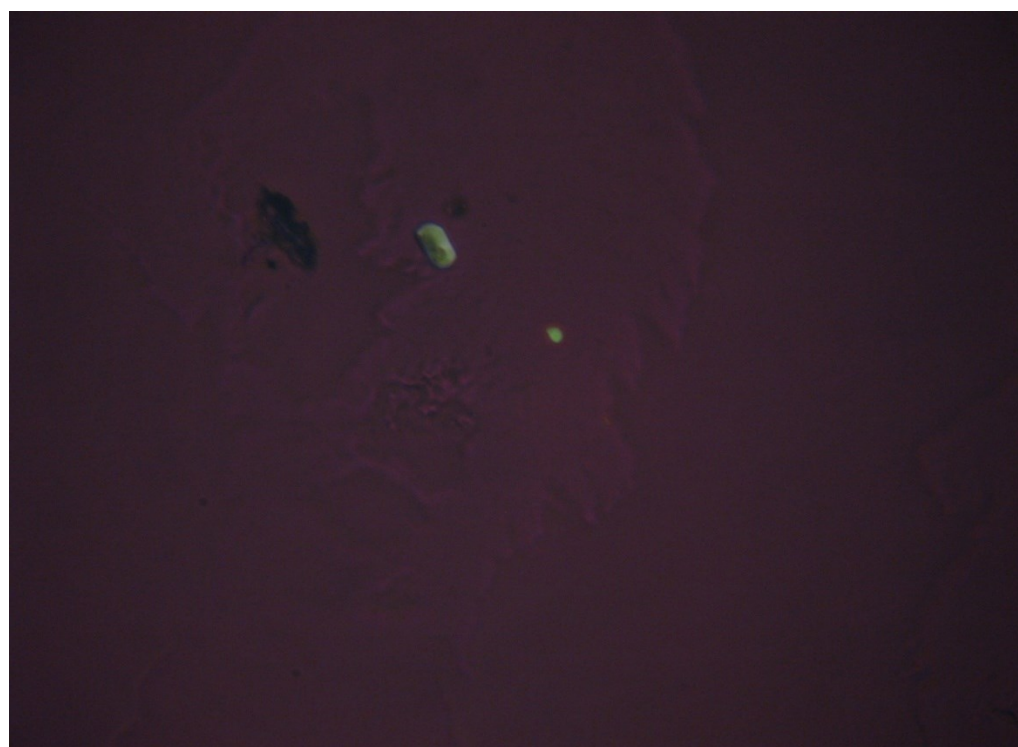


Figure 4. Calcium pyrophosphate crystals observed under polarized light microscopy, x63

One practical point worth emphasizing is that crystal location may be as informative as crystal morphology. A 2025 study by Harris and colleagues showed that intracellular CPP crystals are more strongly associated with acute or chronic CPP crystal arthritis than extracellular crystals alone. This suggests that, when possible, microscopic reports should specify whether CPP crystals are intracellular or extracellular [21].

4.3 Basic calcium phosphate crystals

BCP crystals, including hydroxyapatite, octacalcium phosphate, and tricalcium phosphate, are clinically important but often underrecognized. They have been associated with osteoarthritis, calcific periarthritis, calcific tendinitis, and destructive arthropathies such as Milwaukee shoulder syndrome [1,13,22]. In addition, BCP crystals can induce synovial inflammation through activation of macrophages and the release of pro-inflammatory mediators, contributing to cartilage degradation in osteoarthritis [22].

The main practical challenge is detection. BCP crystals are very small, often submicroscopic, and non-birefringent, which means they are usually not visible with standard CPLM [1,15]. Instead of appearing as well-defined birefringent structures, they may present as amorphous aggregates and often require specialized techniques such as electron microscopy, spectroscopic methods, or alizarin red staining for reliable identification [11,13,15]. For this reason, BCP crystals are more often considered clinically relevant but technically difficult to detect, rather than routinely identified in everyday practice [1,15]. Nevertheless, they remain important, as they broaden the spectrum of crystal-induced joint disease and help explain why a microscopy-negative sample does not necessarily exclude crystal-related pathology [1,13,15].

4.4 Cholesterol crystals

Cholesterol crystals are much less common than MSU and CPP crystals in routine practice, but they are a recognized finding in synovial fluid and should be considered in a full evaluation [5,10]. Morphologically, they appear as large, flat, rectangular plates with characteristic notched corners on light microscopy and show birefringence under polarized light. They are most often seen in long-standing inflammatory joint diseases, particularly chronic rheumatoid arthritis and persistent joint effusions. Although they are not typically associated with acute crystal arthritis, their presence may

reflect chronic synovial inflammation or prolonged joint effusion, and recognizing them helps avoid misclassification [5,10].

4.5 Maltese cross birefringence

Maltese cross birefringence in synovial fluid describes a distinctive optical phenomenon seen under CPLM, most commonly generated by lipid-containing spherical particles. This appearance results from the radial arrangement of lipid molecules within these droplets, producing multiple optical axes and a symmetrical cross-shaped pattern during light extinction [23]. These lipid droplets are typically positively birefringent and may be identified both freely in the fluid and within phagocytic cells, which supports an endogenous origin rather than external contamination [24]. Although not frequently encountered, Maltese cross-like structures have been reported in association with inflammatory joint conditions and can resemble pathological crystals, making accurate identification essential to prevent misdiagnosis [24]. Their presence is thought to be related to processes such as cellular membrane disruption, intra-articular bleeding, or the release of lipids into the joint space, and they may appear in the setting of acute inflammation even when systemic lipid levels are unremarkable [23] (Figure 5).

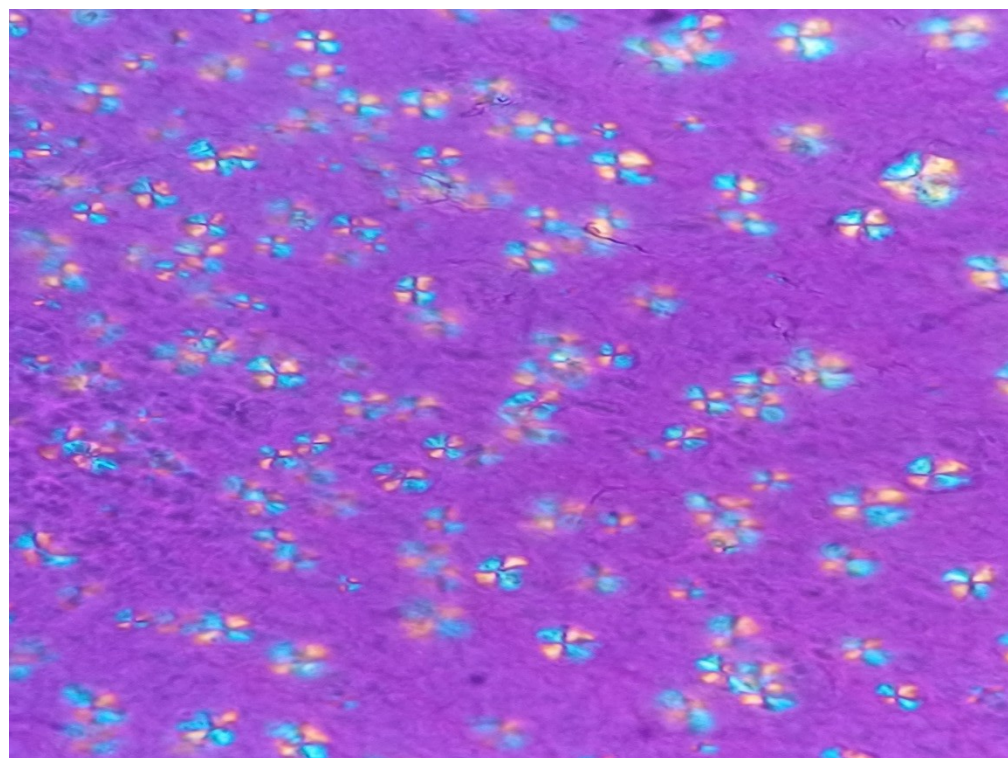


Figure 5. Maltese cross birefringence observed under polarized light microscopy, x63.

More advanced analytical methods, including Raman microspectroscopy and electron microscopy, have confirmed their lipid nature and helped distinguish them from monosodium urate and CPP crystals [25]. In clinical practice, while these structures are not specific to a single disease entity, their recognition remains important for the correct interpretation of synovial fluid findings.

4.6 Other uncommon crystals and particles

Recent reviews of synovial fluid analysis also describe a range of less common findings, including calcium oxalate crystals, corticosteroid crystals following intra-articular injection, lipid droplets, phospholipid microspherules, fibrin aggregates, and cartilage or meniscal debris [5]. These are important because they can mimic true crystals or make microscopic interpretation more difficult, particularly in samples with mixed particulate material [5].

Calcium oxalate crystals are particularly relevant in patients with renal failure or those on haemodialysis. These patients often have elevated plasma oxalate levels due to impaired renal clearance and dialysis does not fully normalize oxalate concentrations [26]. Persistent hyperoxalemia can lead to calcium oxalate crystal deposition in tissues and contribute to systemic oxalosis, including musculoskeletal involvement [27].

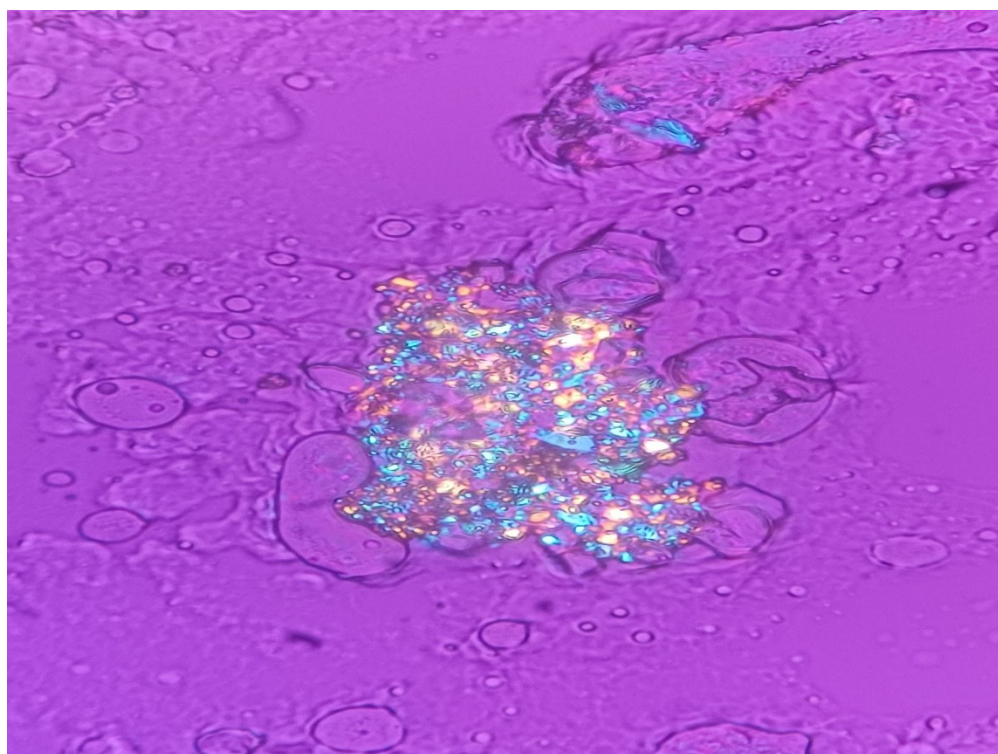


Figure 6. Corticosteroid crystals following intra-articular injection, x63.

Corticosteroid crystals are another practical consideration, as they may appear after intra-articular injections and can be mistaken for endogenous crystals if the clinical context is not taken into account [5].

4.7 Practical considerations in microscopic crystal identification

In routine clinical practice, MSU crystals are generally easier to recognize than CPP crystals, as they are strongly birefringent and classically needle-shaped, whereas CPP crystals have weaker optical properties and more variable morphology [2,10,15]. BCP crystals are usually not visible with standard microscopy, while cholesterol crystals, when present, are morphologically distinctive [2,5]. These differences are clinically important, as the performance of synovial fluid microscopy depends not only on crystal burden but also on how easily a given crystal type can be detected.

Crystal identification should not be seen as a purely mechanical process. Accurate interpretation depends on technical quality, observer experience, and awareness of potential artifacts and non-crystalline mimics [2,5,15]. The presence of intracellular crystals, particularly within neutrophils, supports active inflammation, both in gout and, based on more recent evidence, in CPP crystal arthritis [2,11,25]. At the same time, the absence of visible crystals does not exclude crystal-related disease, especially in cases of BCP deposition or technically challenging samples [1,10,27].

The main morphological and optical characteristics of the principal crystal types identified in synovial fluid are summarized in Table 1.

Table 1. Characteristics of crystals identified in synovial fluid.

Crystal type	Morphology	Birefringence (CPLM)	Typical associated diseases	Additional characteristics	Clinical Relevance	Detection method
Monosodium urate (MSU) [2,6,8,9,15]	Needle-shaped crystals	Strong negative birefringence (yellow when parallel, blue when perpendicular to compensator)	Gout	Often found intracellularly within neutrophils during acute gout attacks	Confirms gout; guides urate-lowering therapy	CPLM (gold standard)
CPP [2,11,17,15, 19]	Rhomboid or rod-shaped crystals	Weak positive birefringence (blue)	CPPD, pseudogout	Birefringence may be weak and	Supports diagnosis of CPPD and helps	Polarized light microscopy

		when parallel, yellow when perpendicular)		difficult to detect	differentiate from gout or osteoarthritis	
Basic calcium phosphate (BCP) [1,13,21,29]	Very small, amorphous or spherical aggregates	Non-birefringent	Osteoarthritis, calcific tendinitis, Milwaukee shoulder syndrome	Often requires electron microscopy or spectroscopic methods for detection	May contribute to degenerative and destructive joint disease despite being difficult to identify microscopically	Electron microscopy, spectroscopy, alizarin red staining
Cholesterol crystals [5,11]	Large rectangular plates with	Strong birefringence	Chronic inflammatory arthritis,	Associated with chronic synovial	Suggests chronic synovial inflammation	Light microscopy, polarize

Abbreviations: BCP, basic calcium phosphate; CPLM, compensated polarized light microscopy; CPP, calcium pyrophosphate; CPPD, calcium pyrophosphate deposition; MSU, monosodium urate.

5. Detection methods

5.1 Synovial fluid aspiration and sample handling

Joint aspiration remains the starting point for crystal diagnosis. The reliability of microscopy depends not only on technical expertise, but also on proper pre-analytical handling. As highlighted in Freemont’s atlas [3], key practical aspects include prompt examination, appropriate anticoagulant use, avoidance of crystal-like contaminants, and the value of wet preparations and cytocentrifuge techniques in routine practice.

Specimen handling is critical, as delays, clotting, degeneration, and contamination can all reduce interpretability. Recent studies and methodological reviews continue to highlight pre-analytical factors as an important source of diagnostic error, particularly in samples with low crystal burden or abundant artifacts [6,28].

5.2 Compensated polarized light microscopy

Microscopic examination remains central to synovial fluid analysis. Routine evaluation typically includes macroscopic assessment, leukocyte count, and

direct examination of fresh fluid preparations. The “wet preparation” technique allows identification of cellular elements, crystals, fibrin, lipid material, and other particulate components under conventional light microscopy. Importantly, many diagnostic clues come from this broader assessment of the sample, not just from birefringent crystals [3,5,8].

Compensated polarized light microscopy (CPLM) is the key method for crystal identification, allowing differentiation based on morphology and birefringence. It is direct, relatively fast, inexpensive, and widely available in routine practice [6].

However, its performance is operator-dependent. Berendsen and colleagues showed that identification of non-MSU crystals and recognition of artifacts may be less reliable than often assumed, highlighting the importance of training and experience in synovial fluid microscopy [2]. More recent studies, however, are more reassuring. Bernal and colleagues reported good agreement between observers, particularly among experienced examiners, supporting the continued reliability of CPLM in clinical practice [15].

Taken together, these findings suggest that synovial fluid microscopy should not be replaced, but rather standardized and supported by appropriate training to improve diagnostic accuracy and reproducibility [2,15].

5.3 New methods

Among emerging methods, Raman spectroscopy is one of the most promising. Reviews published since 2019 highlight its ability to identify biochemical signatures of crystals in synovial fluid and related tissues [29]. A key recent advance is the 2025 study by Niessink and colleagues, which showed that machine learning analysis of Raman spectra in a point-of-care setting can achieve high accuracy in classifying both CPPD and gout, offering a more objective alternative to purely visual microscopy [30].

The main advantage of Raman-based methods is that they classify crystals based on composition rather than relying only on morphology and birefringence. This can be particularly useful in mixed-crystal samples and for detecting non-birefringent particles. However, these techniques are not yet part of routine practice in most centers and currently serve more as adjuncts or future directions rather than replacements for standard microscopy [28,29,30].

Fourier-transform infrared (FTIR) spectroscopy and electron microscopy are mainly used in specialist or research settings. Their main strength is that they can identify crystals based on chemical composition or ultrastructure, which is particularly useful for difficult particles such as BCP crystals. However, their use is limited by cost, equipment requirements, and availability [15].

The methods for detection of synovial fluid crystals are presented in Table 2.

Table 2. Methods for detection of synovial fluid crystals.

Method	Principle	Advantages	Limitations	References
Polarized light microscopy	Identification based on morphology and birefringence	Rapid, widely available, gold standard	Observer dependent	[2,8,15]
Conventional light microscopy	Visualization of particles and cells	Useful screening method	Cannot reliably classify crystal type	[3,5,16]
Raman spectroscopy	Chemical identification via spectral signature	High specificity	Limited availability	[29,34]
FTIR spectroscopy	Infrared spectral analysis of crystal composition	Accurate chemical identification	Specialized equipment required	[28,29]
Electron microscopy	High-resolution structural analysis	Detects very small crystals	Expensive and not routinely available	[11,31]

Abbreviations: FTIR, Fourier-transform infrared.

6. Diagnostic pitfalls in crystal identification

Crystal identification is not error-free and its main pitfalls are well recognized. Weak birefringence and low crystal burden can lead to false-negative results, particularly in CPPD. Conversely, artifacts and non-crystalline particles may result in false positives. Variability in observer experience has also been shown to significantly affect the accuracy of synovial fluid interpretation, highlighting the need for standardized training and quality control in microscopy [5]. Common sources of misinterpretation include fibrin fragments, cartilage debris, steroid crystals, dust, anticoagulant-related contamination, and other foreign material [2,5,16,28].

Mixed-crystal samples present an additional diagnostic challenge. The presence of one obvious crystal type may distract from another, and BCP crystals may be missed entirely if interpretation relies only on

birefringence. Inadequate training also remains a persistent issue, with recent methodological reviews emphasizing that many errors are related to human factors rather than conceptual limitations, reinforcing the need for ongoing training and standardization [2,15,28].

In addition, improper specimen handling can affect accurate crystal identification. Synovial fluid samples should ideally be examined promptly after aspiration, as delays may lead to cellular degradation and changes in the physical properties of the fluid. Careful attention to these factors, together with appropriate training in synovial fluid microscopy, is essential for accurate crystal identification and reliable diagnosis of crystal-induced arthropathies. In more difficult cases, the findings should always be interpreted in the clinical context, and additional laboratory or imaging studies may be needed to confirm the diagnosis of crystal-induced arthritis.

7. Clinical implications of crystal identification

The most immediate implication of crystal identification is diagnostic certainty. In suspected gout, the presence of MSU crystals confirms the diagnosis [8]. In suspected CPPD, identification of CPP crystals not only supports the diagnosis but also aligns with current classification criteria [17].

Synovial fluid analysis is not only confirmatory, but can also influence the diagnostic process. Zamudio-Cuevas and colleagues reported that it led to a change in the initial diagnosis in a meaningful proportion of cases, highlighting that aspiration can actively guide clinical reasoning [31].

This is particularly relevant in CPPD, which often overlaps with osteoarthritis, seronegative rheumatoid arthritis, and other chronic inflammatory phenotypes. Application studies of the 2023 ACR/EULAR classification criteria suggest that CPPD may be underrecognized in patients previously labeled as having other inflammatory arthritides [32].

One of the most important clinical messages in recent literature is that the presence of crystals does not exclude septic arthritis. Mukerji and colleagues reported clinically significant rates of concomitant septic and crystal arthritis in a 10-year retrospective study, while Pesante and colleagues showed that crystal presence can alter the predictive value of standard synovial markers in suspected septic arthritis [20,31].

Therefore, crystal-positive synovial fluid should still be cultured and interpreted in clinical context whenever infection is suspected. Overlooking this is a major practical error in the assessment of acute arthritis.

In patients presenting with acute or chronic arthritis, synovial fluid aspiration remains the first-line diagnostic procedure. Initial synovial fluid analysis includes macroscopic examination, leukocyte count, Gram stain, and culture to exclude septic arthritis. Microscopic examination using compensated polarized light microscopy allows identification of crystals such as MSU and CPP. If crystals are identified, the diagnosis of crystal-induced arthritis can be established in the appropriate clinical context. When microscopy is inconclusive or aspiration is not feasible, complementary imaging techniques such as ultrasound or DECT may assist in detecting crystal deposition. Emerging technologies including Raman spectroscopy and other spectroscopic techniques may further enhance crystal identification and characterization in the future. The diagnostic approach to suspected crystal-induced arthritis is shown in Figure 7.

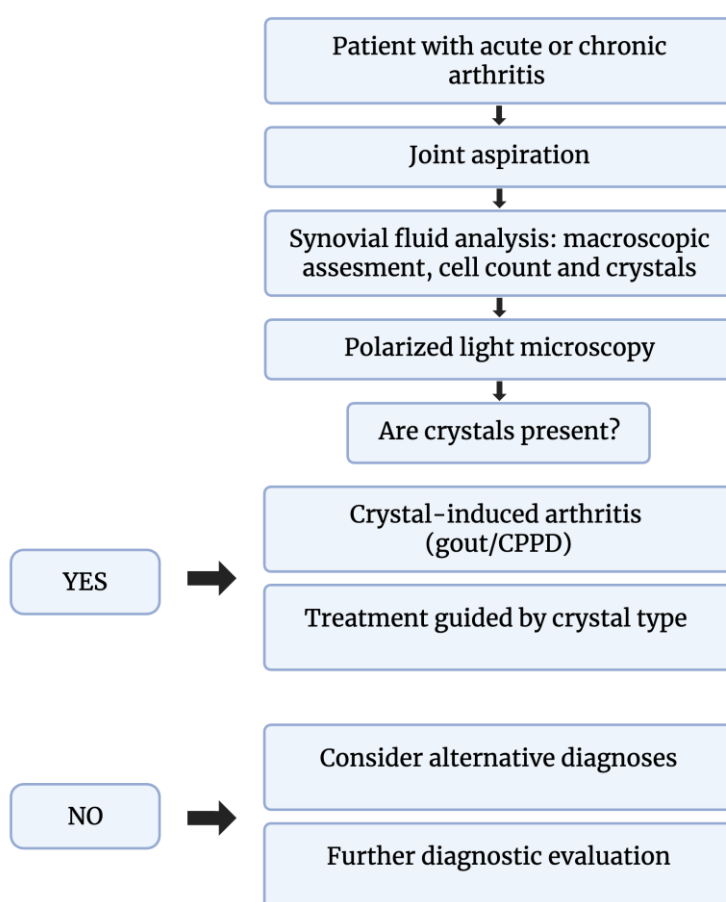


Figure 7. Diagnostic approach to suspected crystal-induced arthritis.

8. Imaging as a complement to synovial fluid analysis

Imaging has advanced significantly in recent years. The 2023 EULAR imaging recommendations, along with their supporting systematic review, highlight that ultrasound, CT, and dual-energy CT can provide valuable information on crystal deposition, inflammation, and structural damage in crystal-induced arthropathies [1,31].

Ultrasound is particularly useful in gout and CPPD, while DECT is especially valuable for urate detection. However, imaging remains complementary. It cannot replace microbiological analysis and does not provide the same direct confirmation of the causative crystal that synovial fluid aspiration offers, especially in diagnostically challenging cases of acute monoarthritis [1,11].

These modalities are particularly helpful when synovial fluid aspiration is not feasible or yields inconclusive results [1,32]. However, imaging remains complementary, as it cannot provide definitive crystal identification or exclude septic arthritis. Therefore, imaging findings should always be interpreted in conjunction with synovial fluid analysis rather than as a substitute for it.

9. Discussion

This review highlights that SF crystal analysis remains a cornerstone of diagnosis in crystal-induced arthropathies, despite significant advances in imaging and emerging spectroscopic technologies. Across contemporary literature, there is strong consensus that direct identification of crystals in synovial fluid continues to provide the highest diagnostic specificity for gout and CPPD. It remains central to clinical decision-making in acute and chronic arthritis [8,9,17].

A key observation from recent studies is that the diagnostic value of synovial fluid extends beyond crystal detection, but arises from its ability to integrate cellular, biochemical, and particulate information reflective of the intra-articular environment [3,16]. While traditional classification systems have focused on distinguishing inflammatory from non-inflammatory effusions, modern evidence reinforces that synovial fluid acts as a direct surrogate of ongoing joint pathology, particularly in crystal-driven inflammation where innate immune activation is central [7].

MSU crystals remain diagnostically definitive for gout due to their characteristic morphology and strong negative birefringence under CPLM

[8,9]. However, current literature also emphasizes that diagnostic certainty in gout is not solely dependent on clinical phenotype or serum urate levels, which may be normal during acute flares [6]. This reinforces the continued necessity of synovial fluid examination as the reference standard rather than an adjunctive test.

In CPPD, diagnostic challenges are more pronounced. CPP crystals exhibit weaker birefringence and greater morphological variability, which contributes to reduced detection reliability compared with MSU crystals [11,15]. The incorporation of CPP crystal identification into the 2023 ACR/EULAR classification criteria underscores its diagnostic importance, but also reflects a broader shift toward recognizing CPPD as a heterogeneous disease spectrum rather than a single clinical entity [17]. Recent studies further suggest that intracellular localization of CPP crystals may correlate more strongly with clinically active disease, indicating that not only crystal presence, but also distribution, may carry diagnostic significance [21].

Basic calcium phosphate (BCP) crystals represent an additional diagnostic challenge. Their submicroscopic size and non-birefringent nature make them largely invisible under standard CPLM, requiring specialized techniques such as electron microscopy or spectroscopic analysis for detection [11,13]. Despite these limitations, BCP crystals are increasingly recognized as important contributors to osteoarthritic and periarticular disease, highlighting an ongoing diagnostic limitation in routine clinical practice [22].

An important clinical issue is the coexistence of crystal-induced arthritis with septic arthritis. Recent evidence demonstrates that the presence of crystals does not exclude infection, and dual pathology may occur more frequently than previously appreciated [20,31]. This has important implications for clinical practice, as reliance on crystal identification alone may lead to misclassification of septic arthritis as purely inflammatory disease. Therefore, microbiological culture remains essential in all cases of suspected joint infection, regardless of crystal findings [31].

From a methodological perspective, compensated polarized light microscopy remains the most widely used and practical diagnostic tool, but its accuracy is influenced by several factors including observer experience, sample quality, and pre-analytical handling [2,5,28]. While earlier studies raised concerns regarding variability and interpretive error [2], more recent work suggests that structured training significantly improves interobserver reliability, supporting microscopy as a robust but skill-

dependent technique rather than an outdated modality [15]. This distinction is important, as diagnostic failure is more often attributable to human and pre-analytical factors than to intrinsic limitations of the method itself.

An additional practical limitation is the lack of universally standardized training protocols and competency assessment for synovial fluid crystal identification across laboratories. This variability may contribute to differences in diagnostic reproducibility and highlights the need for more uniform educational and quality-control approaches in routine clinical practice [2,15,28].

Emerging technologies such as Raman spectroscopy and FTIR spectroscopy offer promising advances by enabling crystal identification based on chemical composition rather than morphology alone [13,29]. The integration of machine learning with Raman spectral analysis further improves classification accuracy for MSU and CPP crystals, suggesting a potential future shift toward automated or semi-automated diagnostic workflows [30]. However, despite their high analytical potential, these technologies remain limited by cost, availability, and lack of widespread clinical implementation, and therefore currently function as complementary rather than replacement tools [28,30].

Another potential future direction is the development of artificial intelligence (AI)-assisted microscopy and automated image analysis systems for synovial fluid examination. Such approaches may help reduce observer dependence and improve reproducibility in crystal identification, particularly for weakly birefringent or low-burden samples. AI-supported analysis could also assist less experienced observers and contribute to greater standardization of synovial fluid microscopy in routine clinical practice [28,30]. Although these technologies remain largely investigational, their integration with spectroscopic and machine learning-based methods may further enhance the accuracy and objectivity of crystal detection in the future [29,30].

Imaging modalities, particularly ultrasound and DECT, have expanded the non-invasive detection of crystal deposition and structural joint changes [1,31]. Ultrasound is especially useful in detecting both gout and CPPD-related changes, while DECT provides high specificity for urate deposition. Nevertheless, imaging lacks the ability to provide definitive etiological confirmation or exclude infection, and therefore cannot replace synovial fluid analysis in diagnostic pathways [1,32]. Its role is best understood as

complementary, particularly in cases where aspiration is not feasible or results are inconclusive.

Overall, the most important conceptual development in recent literature is the recognition that diagnostic uncertainty in crystal arthropathies is no longer primarily due to inability to detect crystals, but rather due to variability in interpretation, overlapping clinical phenotypes, and coexistence with other joint pathologies. In this context, synovial fluid analysis retains its position as the most direct and clinically decisive diagnostic tool, provided its limitations are acknowledged and appropriate methodological standards are maintained.

Future progress is likely to depend less on replacing microscopy and more on improving its reproducibility, integrating it with objective analytical techniques, and enhancing clinician training. Until such advancements are fully realized, synovial fluid examination remains an indispensable component of rheumatologic diagnosis, combining speed, accessibility, and direct etiological confirmation in a way that no current alternative method can fully replicate.

10. Conclusions

Crystals in synovial fluid remain central to the diagnosis of crystal-induced arthropathies. MSU and CPP crystals are the most clinically important and diagnostically actionable, while BCP and cholesterol crystals broaden the differential and contribute to a fuller understanding of joint pathology. Compensated polarized light microscopy remains the practical gold standard and its accuracy depends on appropriate sample handling and observer expertise. Newer objective methods, especially Raman spectroscopy, are promising and may reduce observer dependence in the future. Imaging has become an important complement, but not a replacement, for aspiration-based diagnosis. Most importantly, crystal identification must always be interpreted in clinical context, particularly because crystal arthritis and septic arthritis can coexist. In that sense, synovial fluid analysis remains not an old-fashioned test, but one of the most clinically decisive investigations in rheumatology.

Declarations

Author Contributions

Conceptualization: V.T.; Methodology: M.I., V.T.; Writing - original draft preparation: V.T.; Writing - review and editing: M.I., G.K., V.T.

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The authors declare no conflict of interest.

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