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Point-of-care devices for the detection of biomarkers of periprosthetic joint infection: State of the art and future perspectives

Vincenzo Mazzaracchio [a,b], Raffaele Vitiello [c,d], Giulio Maccauro [c,d], Fabiana Arduini [a,b,*]

[a] Department of Chemical Science and Technologies, University of Rome "Tor Vergata", Via Della Ricerca Scientifica, 00133, Rome, Italy
[b] SENSE4MED, Via Bitonto 139, 00133, Rome, Italy
[c] Department of Geriatrics and Orthopaedic Sciences, Università Cattolica Del Sacro Cuore, Rome, Italy
[d] Department of Aging, Neurological, Orthopaedic and Head-Neck Sciences, Fondazione Policlinico Universitario Agostino Gemelli Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS), Rome, Italy

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ABSTRACT

One of the main issues in the management of periprosthetic joint infection (PJI) is related to the correct diagnosis. Current guidelines for PJI infection are based on the 2018 Philadelphia Consensus Criteria which encompasses major and minor criteria, where minor criteria are based on the detection of selected biomarkers in synovial fluid or serum samples. In 2021, the European Bone and Joint Infection Society revised the aforementioned criteria; however, current methods require a long analysis time. In this overall scenario, we report the state of the art and the recent advances of point-of-care devices and implantable sensors for a new diagnostic approach in the diagnosis of PJI by quantifying well-established and emerging biomarkers in serum, blood, and synovial fluid. Finally, future challenges and perspectives have been reported, highlighting the relevance of sensing devices for paving a new concept of diagnosis and monitoring in the PJI field to solve this important issue.

1. Introduction

In 2016–2017, the European Centre for Disease Prevention and Control conducted a survey to collect data on healthcare-associated infections in European countries, estimating a total of 8.9 million healthcare-associated infections each year, where 4.5 million were reported in hospitals and 4.4 million in long-term care facilities [1]. Among healthcare-associated infections, the use of prosthetic devices in surgical procedures highlighted their liability for the risk of infection [2–5]. Indeed, despite following the best practices in surgical management using prophylaxis and antibiotics treatments, these infections have a significant burden on the healthcare expenditure and the quality of life of the patients. This is mainly caused by the presence of bacteria resistant to the few antibiotics with proven anti-biofilm activity, including Rifampicin-resistant staphylococci, Ciprofloxacin-resistant Gram--negatives, and Methicillin-resistant *S. aureus*. For instance, in Italy it has been reported that 1.55 % of the total hospitalizations for prosthetic implants in 2014 were associated with a diagnosis of infection, with a total cost of ca. EUR 50 million [6], while methicillin-resistant *S. aureus* infections are estimated to affect more than 150,000 patients annually in the European Union (EU) with costs of EUR 380 million for EU healthcare systems [7].

The predisposition and the increased susceptibility of orthopedic device-related infections are mainly regulated by two factors, namely the ability of bacteria to form biofilm on/around the implanted device and the deficit in host immunological defenses close to the device. Fintan Moriarty et al. [8] highlight that the risk of infection can be considered universal for all implanted orthopedic devices, caused by the presence of biofilm which grows on each implant. Indeed, the biofilm hinders the effect of antibiotics by protecting the bacteria from host phagocytes and reducing the metabolic activity of bacteria.

One of the main issues for the sustainable management of periprosthetic joint infection (PJI) is related to the correct diagnosis. International Consensus Meeting (ICM) on musculoskeletal infection first proposed its diagnostic criteria in 2013 including major and minor criteria [9] (Table 1). In detail, major criteria encompass two positive periprosthetic cultures with phenotypically identical organisms or a sinus tract communicating with the join. Minor criteria comprise elevated serum *C*-reactive protein (CRP) >10 mg/dL for acute PJI and >1 mg/dL for chronic PJI; erythrocyte sedimentation rate (ESR) > 30

* Corresponding author. Department of Chemical Science and Technologies, University of Rome "Tor Vergata", via della Ricerca Scientifica, 00133, Rome, Italy.
E-mail address: Fabiana.arduini@uniroma2.it (F. Arduini).

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Table 1
Minor criteria biomarker levels for PJI diagnosis stated during the years.

	Minor Criteria Biomarker	2013 International Consensus Meeting on musculoskeletal infection [9]		2018 [12]		2021 European Bone and Joint Infection Society [13]		
		Chronic (>90 days)	Acute (<90 days)	Chronic (>90 days)	Acute (<90 days)	Infection Unlikely	Infection likely	Infection confirmed
SERUM	ESR (mm/hr)	30	–	30	–	–	–	–
	CRP (mg/dL)	1	10	1	10		>1	
	D-dimer (μg/L)	–	–	860	860[a]	–	–	–
SYNOVIAL FLUID	WBC count (cells/μL)	3000	10,000	3000	10,000	≤1500	>1500	>3000
	Leukocyte Esterase	+ or ++	+ or ++	+ or ++	+ or ++	–	–	–
	PMN (%)	80	90	80	90	≤65	>65	>80
	CRP (mg/L)	–	–	6.9	6.9[a]	–	–	–
	Alpha-defensin (signal-to-cut-off ratio)	–	–	1	1	–	–	Positive immunoassay or lateral flow

CRP: *C*-reactive protein; ESR: erythrocyte sedimentation rate; PMN: polymorphonuclear; WBC: white blood cell.
[a] Further studies are needed to validate a specific threshold.

mm/h only in chronic PJI; a single positive culture; elevated synovial fluid white blood cell (WBC) count >10.000 cell/μL in acute PJI and >3.000 cell/μL in chronic PJI or positive change on leukocyte esterase (LE) test strip (+ or ++ on colorimetric strip); elevated synovial fluid polymorphonuclear neutrophil percentage (PMN%) > 90 % in acute PJI and >80 % in chronic PJI; positive histological analysis of periprosthetic tissue with >5 neutrophils per high power field in 5 high power fields (x400). In the case of minor criteria, the detection of biomarkers is assessed in two types of matrices, namely serum and synovial fluid. If the serum is a well-established matrix in all clinical practices, the synovial fluid is a matrix mainly selected and analyzed in PJI. Synovial fluid is a viscous solution present in the cavities of synovial joints with the principal function of reducing friction between the articular cartilages of synovial joints during movement [10]. This matrix is composed of blood plasma ultrafiltrate including proteins and additional molecules such as hyaluronan, proteoglycan, cytokines, and metabolic byproducts able to modulate synovial inflammation [11]. Considering this feature and that synovial fluid is near the joint tissue, synovial fluid is primarily altered during these articular diseases. Furthermore, synovial fluid can be collected by arthrocentesis, a minimally invasive articular procedure, thus the sampling combined with the informative composition enables its use as a reliable matrix for PJI diagnosis.

In this overall scenario, in 2018, minor criteria were updated, by dividing these biomarkers into serum-derived and synovial fluid-derived [12]. Briefly, major criteria are considered: two positive cultures or a sinus tract presence. However, in the absence of major criteria, minor

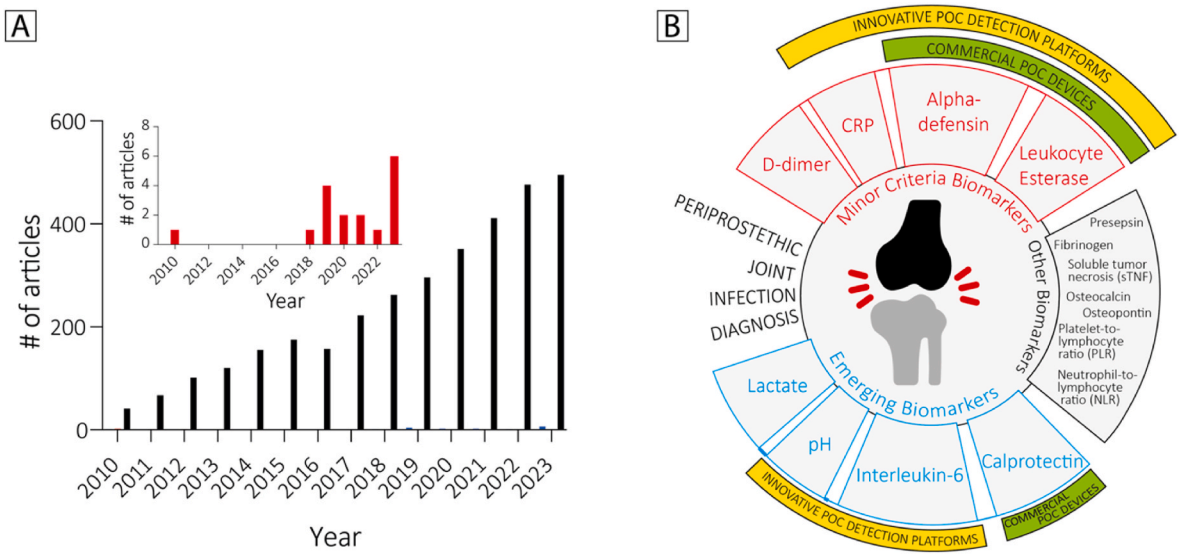

Fig. 1. A) Histogram bars of the publication statistics through the years regarding the topic of PJI diagnosis (black bars: Scopus search using as keywords: "periprosthetic and joint and infection and diagnosis"; inset, red bars: Scopus search using as keywords: "sensors and periprosthetic and joint and infection and diagnosis and synovial and fluid and blood and serum". B) Schematic illustration of the established, alternative and other biomarkers for PJI diagnosis showing the presence of commercial/innovative POC detection tools described in this review. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

criteria are considered as follows: 2 points for a serum CRP >1 mg/dL; 2 points for D-dimer >860 ng/mL; 1 point for ESR >30 mm/h; 3 points for a synovial fluid white blood cell count >3000 cells/μL; 3 points for an increased synovial fluid alpha-defensin (signal-to-cut-off ratio >1); 3 points for an elevated synovial fluid leukocyte esterase (++); 2 points for PMN >80 %; and 1 point for synovial CRP >6.9 mg/L. A variable score (from 1 to 3) has been assigned to each criterion and the diagnosis of infection is set for a score greater than or equal to 6 points. For scores between 2 and 5, the diagnosis of "possible infection" is made. Criteria based on intraoperative sampling have also been introduced.

Successively, in 2021 the European Bone and Joint Infection Society (EBJIS) published its variation of the diagnostic criteria for PJI [13]. The new 2021 classification revised the cut-offs and ranges of the selected biomarkers and introduced new methods such as sonification and nuclear radiology. For confirmed infection, one of the following criteria must be present: sinus tract presence; synovial fluid white blood cell count >3000 cells/μL or PMN >80 %; increased synovial fluid alpha-defensin (positive immunoassay or lateral-flow assay); two or more positive samples with same microorganism; >50 CFU/mL of any organism after sonication; presence of five or more neutrophils in more five or more high-power field (400× magnification) or the presence of visible microorganisms in histology. The possible infection diagnosis was established considering two of the following criteria: radiological signs of loosening; previous wound healing problems; history of recent fever; purulence around the prosthesis; CRP >1 mg/dL; synovial fluid white blood cell count >1500 cells/μL or PMN >65 %; positive culture in aspiration fluid; single positive culture from intraoperative fluid or tissue; >1 CFU/mL of any organism after sonication; presence of five or more neutrophils in one high-power field (400× magnification); positive WBC scintigraphy.

In a recent paper by Sigmund et al. [14] the superiority in sensitivity and specificity of the EBJIS criteria compared to the previous ones has been demonstrated.

The need for several parameters and the timely update of parameters highlights the difficulty in PJI diagnosis. In this regard, in 2022 Papalini et al. [3] recommended the use of all the diagnostic tests available to approach PJI diagnosis, suggesting caution before rejecting PJI diagnosis. This caution is ascribed to the fact that it is well-known that current diagnostic methods reach up to 34 % false-negative cultures [4]. Furthermore, it is necessary to have the diagnosis of PJI as well as know the pathogens, because e.g., the decision for debridement and retention of knee PJIs should also depend on the pathogen.

The relevance of diagnostics in PJI diagnosis is emphasized in the increasingly published studies related to PJI in the last decade, including medical-related and diagnostic-based publications (Fig. 1A) and sensors for PJI diagnosis based on the detection of biomarkers in serum or synovial fluid (Fig. 1A, inset).

Additionally, several reviews recently published have highlighted the need for additional diagnostic tools for prompt PJI diagnosis, facing the advantages and disadvantages of well-established laboratory-based analytical techniques, such as molecular diagnostic methods, imaging techniques, microbiological, histological tests, and the measurement of biomarkers in serum/synovial fluid samples [15–22].

Beyond the reviews reported in the literature, this review is devoted to outlining the state of the art, the latest trends, and the opportunities in PJI diagnosis by describing the well-established and innovative diagnostic tools reported in the literature for the detection of biomarkers in PJI diagnosis (Fig. 1B).

The different analytical tools used for established biomarkers as well as for emerging biomarkers were described, highlighting the growing interest in this field. Indeed, if in the first articles the classical lateral flow devices were used, in recent years biosensing scientists have started to develop innovative point-of-care devices for these biomarkers. Furthermore, the latest trends in implantable devices have been reported. In the end, we review the strengthening features of the point-of-care and implantable devices, reporting the prominence of the key technological challenges.

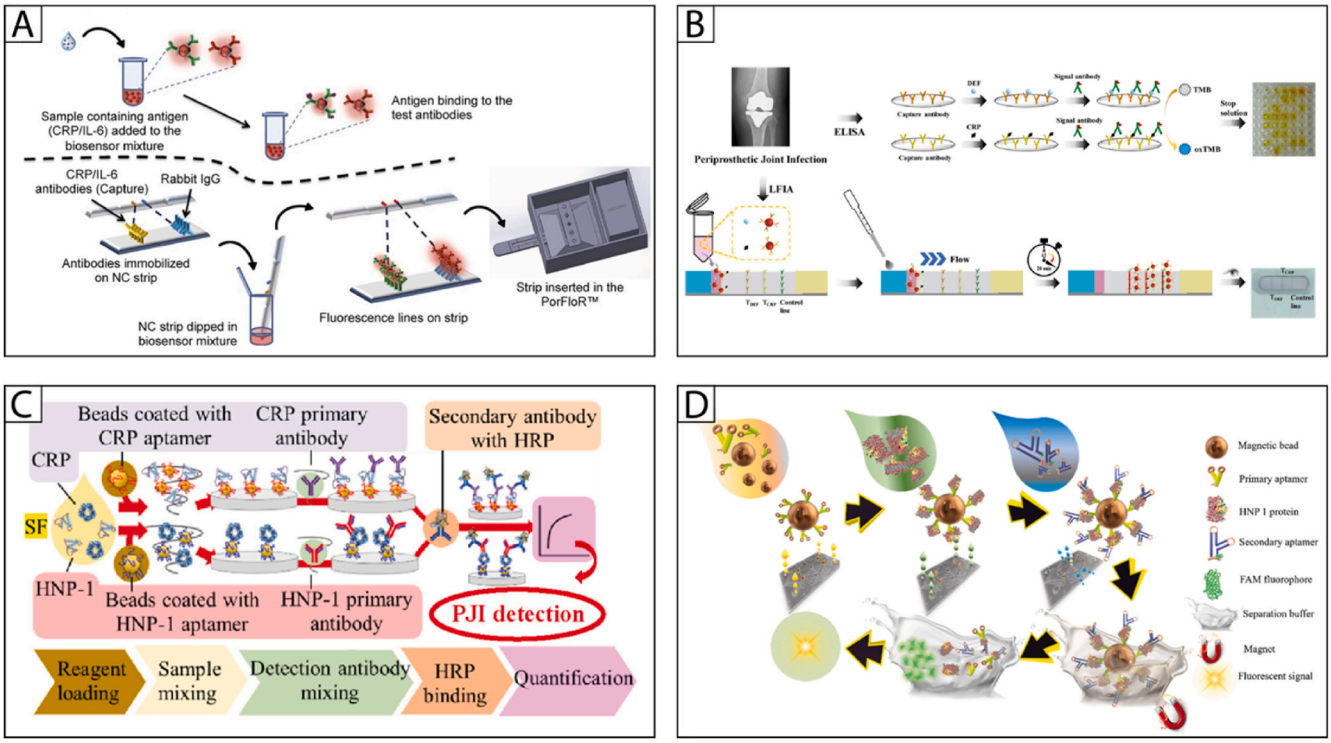

Fig. 2. Periprosthetic joint infection point-of-care devices. A) Fluorescence lateral flow immunoassay device for CRP detection [32]. B) Multiplex lateral flow immunoassay for CRP and alpha-defensin detection [33]. C) Magnetic bead-based fluorometric assay on an integrated microfluidic system for CRP and alpha-defensin detection [34]. D) Aptamer-based sandwich fluorometric assay for detection of alpha-defensin on a microfluidic platform [35].

2. Minor criteria biomarkers detection

The minor criteria biomarkers include CRP in serum and in synovial fluids, D-dimer in serum, synovial fluid white blood cell count, synovial fluid alpha-defensin, and synovial fluid leukocyte esterase.

Among them, D-dimer and synovial fluid white blood cell count analyses have been mainly carried out using laboratory set-up instruments, such as an immunoturbidimetric assay for the case of D-dimer detection [23–26]. For the other biomarkers, several efforts have been made to use enzyme-linked immunosorbent assay (ELISA) compared to laboratory-based immunoturbidimetry assay for assessing the reliability of the selected biomarkers in different matrices. For instance, Parvizi et al. initially used immunoturbidimetry for the detection of CRP in the synovial fluid to evaluate its effectiveness as a biomarker in PJI diagnosis [27], while later the same authors used both commercial ELISA and laboratory immunoturbidimetry assay for a comparative study of serum *vs* synovial fluid CRP [28]. In detail, single ELISA and multiplex ELISA were used for CRP detection in synovial fluid, while immunoturbidimetry was used on serum CRP. Despite the different matrix, and although both tests predicted the infection, the authors report the superior performances of the multiplex ELISA assay analysis over the clinical hospital laboratory analysis, in terms of i) proportion of positive results in infected patients (namely, sensitivity) equal to 84 % *vs* 76 %, ii) proportion of negative results in non-infected patients (namely, specificity) equal to 97.1 % *vs* 93.3 %), and iii) accuracy (91.5 % *vs* 85.5 %), highlighting the advantages of using multiplex ELISA analyses.

The next step forward has been the use of lateral flow devices. Ahmad et al. performed a meta-analysis of several literature studies regarding the performances of the ELISA-based alpha-defensin test compared to Synovasure™ lateral flow assay kit [29], reporting that the laboratory-based alpha-defensin ELISA test demonstrated higher accuracy for PJI diagnosis when compared with Synovasure™ alpha-defensin test kit, which showed a markedly lower accuracy.

Renz et al. analyzed alpha-defensin in synovial fluid by using a lateral flow test from Zimmer Biomet and leukocyte esterase with the strip test Combur-Test from Roche Diagnostics, reporting that alpha-defensin lateral flow in synovial fluid was rapid and highly specific for diagnosing PJI (>95 %) [30]. As stated by the title of this article "*Alpha-Defensin Lateral Flow Test for Diagnosis of Periprosthetic Joint Infection: Not a Screening but a Confirmatory Test*", these authors have posed a pillar from the diagnostic point of view, indicating that the point-of-care devices are effective in PJI diagnosis.

In this trend, Chisari et al. reported a study for leukocyte esterase quantification using a lateral flow test strip in 259 patients, who underwent a revision for total hip arthroplasty or total knee arthroplasty [31]. The general readout system of these strips can give various results: i) negative (white), ii) presence of traces (slightly purple), iii) positive (usually light purple strip color, commonly referred to as + or 1+), and iv) very positive (usually dark purple, commonly referred to as ++ or 2+). A sensitivity of 74 % and a specificity of 91 % were obtained when a cut-off value $\geq$ 1+ was used for the assessment, while a specificity of 100 % was observed using a cut-off value of 2+. The authors highlighted that the leukocyte esterase strip test yielded a high specificity, positive predictive value, negative predictive value, and moderate sensitivity.

To cope with the moderate sensitivity of colorimetric lateral flow devices, in the last 5 years there has been growing interest in developing more sensitive point-of-care devices by implementing the configuration of diagnostic with microfluidics devices and/or using more innovative (nano)materials such as quantum dots, magnetic beads, and aptamers.

Borse et al. coupled CRP detection with interleukin-6 for the monitoring of both target analytes in serum samples, by developing a customized lateral flow immunoassay [32] (Fig. 2A). In detail, focusing on CRP detection, a nitrocellulose strip was functionalized with a recognizing compound, comprising a staphylococcal protein A conjugated with CdTe quantum dots and antibodies anti-CRP. Once the lateral flow immunoassay was performed by adding the solution containing the target analyte, the fluorescence was evaluated by a portable fluorescence strip reader (PorFloR™). The linear range in standard solution was found to be 1–300 μg/mL, whereas the limit of detection was equal to 0.3 μg/mL. Finally, the developed sensor was used for the analyses of one serum sample spiked with different CRP concentrations. Results were compared with one obtained by the standard ELISA technique, obtaining a satisfying correlation coefficient equal to 0.998.

To improve the sensitivity, ImageJ software or RGB photometric readout was used.

Tsai et al. [36] used the ImageJ software coupled with a CCD camera readout system to perform quantitative measurements using an enhanced LFIA system. In detail, an additional stacking pad was used to improve the binding interactions between antibody and antigen, hence enhancing the test's detection sensitivity. Using the described approach an outstanding detection limit of 15.5 ng/mL was obtained, and analyses in serum and synovial fluid were performed.

The same authors [33] home-fabricated a lateral flow immunoassay to detect in the same analysis both CRP and alpha-defensin by using gold nanoparticles modified with CRP and alpha-defensin antibodies dispensed on different reaction zones in nitrocellulose substrate (Fig. 2B). Focusing on CRP detection, anti-CRP and goat anti-mouse IgG were dispensed on the nitrocellulose substrate to generate the test line and the control line, respectively. The synovial fluid samples collected from patients who underwent total hip or knee replacement were analyzed by the developed lateral flow immunoassay, and the optical intensity of the test strip color was analyzed by ImageJ software. Comparing the recorded data with results obtained by a commercial ELISA kit, an acceptable correlation coefficient equal to 0.92 was observed for the target analyte tested.

Zheng et al. coupled the commercially available colorimetric strips with an RGB photometric readout system [37]. In detail, after performing the assay by the leukocyte esterase test strips, the image was inputted into the RGB photometric system, to obtain the value called "brightness". By correlating through an equation the Red, Green, and Blue color values, the final parameter of "brightness" is obtained. The authors found the lower brightness value associated with the higher activity of leukocyte esterase. Analyzing the synovial fluid of 46 patients with suspected PJI, a good correlation was obtained between the brightness (Y) of LE Strips and WBC count, with an R^2 equal to 0.858. Nevertheless, the proposed approach still shows some drawbacks, mainly connected to the interference from the external (i.e., external light and camera equipment settings) and internal (i.e., red blood cells) environment.

Besides the lateral flow device, an interesting work has been published this year (i.e. 2023) in Analytical Chemistry journal reporting a microfluidic device based on luminescence signal readout for alpha-defensin and CRP detection in synovial fluid [34]. In detail, magnetic beads were modified with capture aptamers, whereas horseradish peroxidase-labeled antibodies were used for the final signal generation after the addition of luminol (Fig. 2C). With this device configuration, simultaneous detection of alpha-defensin and CRP biomarkers was assessed. In detail, for CRP detection a threshold of 6.6 mg/L was observed, while a lower threshold equal to 2.6 mg/L was obtained in the case of alpha-defensin. Finally, synovial fluid from patients with diagnosed PJI was analyzed. The results were consistent with those obtained using the commercial ELISA, with a correlation coefficient equal to 0.993, demonstrating the reliability of aptamer-based microfluidic devices.

Always in 2023, another interesting optical microfluidic platform was reported by Gandotra et al. [35] for the detection of alpha-defensin in synovial fluid samples. The device has been configured using an aptamer-based sandwich assay to detect alpha-defensin by fluorescence measurements, within less than 60 min and a 100 % accuracy. In detail, magnetic beads were coated with a specific aptamer able to bind the target analyte (Fig. 2D). A fluorescent-labeled secondary aptamer was

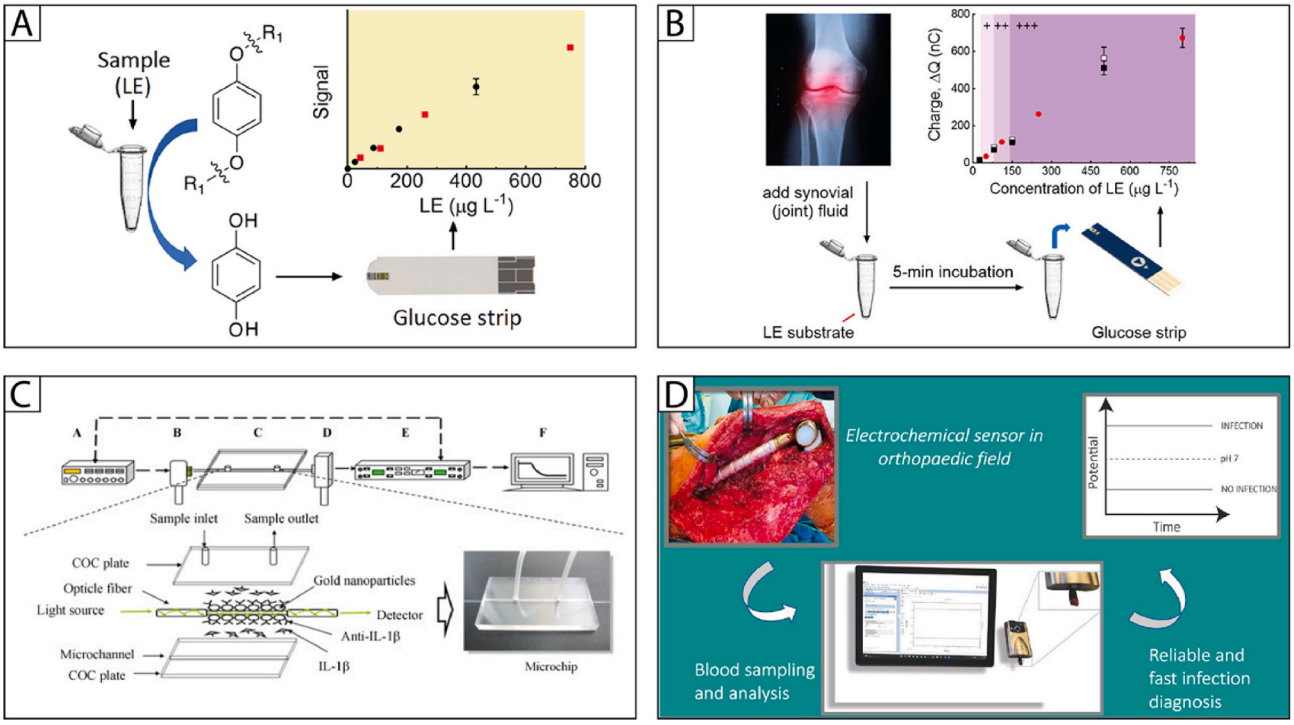

Fig. 3. Periprosthetic joint infection point-of-care devices. A) Coulometric sensor for leukocyte esterase detection by glucose test strips [39]. B) Leukocyte esterase detection by glucose test strips with blood glucometer [40]. C) Fiber-optic particle plasmon resonance sensor for detection of interleukin-1β in synovial fluids [41]. D) Bluetooth-assisted miniaturized electrochemical sensor for pH detection in blood during orthopedic surgery [42].

further used to quantify the alpha-defensin with the sandwich approach, in a linear range of 0.5–100 mg/L. To validate the sensing device, 13 clinical samples were analyzed, without the need for sample pretreatment, obtaining a sensitivity and specificity of 100 % for both parameters, highlighting the reliability of the developed analytical tool.

More recently, the same authors exploit an aptamer-based assay for the quantitative detection of alpha-defensin [38]. In detail, the analytical tool is based on a microfluidic chip for sample management coupled with a nitrocellulose paper-based substrate for aptamer-immobilization. Using a fluorimetric read-out system a limit of detection of 0.5 mg/L was obtained in standard solution, while clinical sample analyses were performed in less than 42 min on a single chip, with 100 % accuracy, as confirmed by comparison with "gold-standard".

Beyond optical point-of-care devices, at the state of the art, two electrochemical point-of-care devices have been developed for leukocyte esterase quantification using commercially available glucose test strips in two different configurations.

In the first configuration, the glucose strips were used in a coulometric assay, using a potentiostat based on laboratory set-up [39]. The measurement relies on the first reaction of leukocyte esterase with an ester containing a hydroquinone main structure (Fig. 3A). After this enzymatic reaction, the released hydroquinone reacts at the glucose test strip surface, by applying an oxidizing potential of 0.4 V. The electrons were quantified by the charge produced, giving the final chronocoulograms for the quantification in the clinically relevant leukocyte esterase concentration range of 20–750 µg/L. Furthermore, commercially available human synovial fluid was spiked with different concentrations of leukocyte esterase, and the obtained measurements proved the reliability of the developed sensing approach in synovial fluid, regardless of the presence of blood (i.e., red blood cells), or color-imparting substances.

In the second configuration, the authors used the glucose strips directly connected to a glucometer [40]. In this study, leukocyte esterase reacts with synthesized esterified glucose molecules, producing not-functionalized glucose, which further reacts with the glucose oxidase enzyme on the surface of the glucose strips (Fig. 3B). The final readout system, namely the glucometer or a potentiostat, gives the quantification of glucose which is proportional to leukocyte esterase content. Compared to classic test strips, the proposed biosensor has the advantage of short sample incubation, and a high-resolution signal regardless of sample condition (turbidity, color). Nevertheless, the subtraction of the signal of the original sample from that of the incubated sample is necessary to eliminate the interferences, including those from glucose.

3. Emerging biomarkers detection

Alongside the biomarkers included as minor criteria by the 2018 ICM, several alternative analytes are gaining high relevance, showing promising potential for accurate PJI diagnosis. Since the threshold levels to diagnose PJI from these biomarkers are not stated, they are usually obtained by statistical analyses and comparison with previous studies.

3.1. *Calprotectin*

Calprotectin is a cytosolic antibacterial heterodimeric protein, contained in the neutrophil cytosol. It is released upon neutrophil activation, as it is involved in the antibacterial defense mechanisms of the immune system [43]. For this reason, high calprotectin levels can be a sensitive non-specific inflammatory marker in various clinical circumstances. In the field of PJI diagnosis, calprotectin has been considered an additional biomarker in synovial fluid, identifying 173 µg/mL [44] or 50 µg/mL as a threshold value [45].

Two main approaches are used for calprotectin detection, namely ELISA and lateral-flow test strip, confirming a comparable sensitivity and specificity to the other well-known biomarkers in PJI diagnosis. ELISA represents the well-established approach for calprotectin detection in synovial fluids [44–47], with sensitivity ranging from 92 % [46] to 100 % [47], and specificity from 95 % [47] to 100 % [46].

Recently, several works have focalized their study on calprotectin

analyses using commercially available lateral-flow test strips able to carry out rapid and user-friendly quantitative measurement by laboratory-based readers or smartphone readers.

As a first approach, Wouthuyzen-Bakker et al. [45] applied the commercial Quantum Blue® quantitative lateral-flow assay, which is developed for fecal calprotectin analysis, for the measurement of the target analyte in synovial fluid. Using this laboratory-based analysis, the synovial fluid from 61 patients (19 with a PJI and 42 controls) was analyzed, obtaining a sensitivity of 89 % and a specificity of 90 %. More recently, multiple studies applied the commercial lateral flow assay Lyfstone AS, specifically developed for calprotectin analysis in synovial fluids. In this case, the smartphone-based app for digital results acquisition allows for rapid semi-quantitative analysis, enabling the measurement directly at the point-of-care [48–50]. Using this sensing device specifically designed for calprotectin analysis in synovial fluids the authors obtain sensitivity and specificity in the range of 71–94 % and 69–81 %, respectively.

3.2. Interleukin-6

Interleukin-6 (IL-6) is a pro-inflammatory protein released in the bloodstream, stimulating the expression of a variety of cytokines responsible for acute inflammation, thus contributing to the defense through the induction of acute phase responses [51].

As an alternative biomarker for PJI diagnosis, the first studies for IL-6 detection were based on the ELISA test in serum samples [52–54]. Nevertheless, using the laboratory-based ELISA technique on serum samples from patients who underwent orthopedic arthroplasty, low sensitivity and specificity were recorded (i.e., in the range of 72–81 % and 63–82 %, respectively), underlying the need for more clinical samples analyses and studies, or the use of different biofluid, i.e., synovial fluid.

Yu et al. [54] compared the detection of IL-6 analyzed in serum and synovial fluid of 151 patients with a possibility of infection after total knee or hip arthroplasties. The ELISA tests showed a serum IL-6 sensitivity of 80.7 % and a specificity of 81.8 %, lower than the synovial fluid IL-6 sensitivity and specificity, equal to 90.3 % and 88.3 %, respectively. The proposed studies report the need for additional testing to enhance the reliability of the IL-6 test for PJI detection, still showing a promising relevance of this biomarker.

For this emerging biomarker, the authors selected a threshold value of 8.98 pg/mL in the serum sample and 6.59 ng/mL in synovial fluid [54]. The value reported as the threshold for serum by Yu et al. [54] is in agreement with other threshold values reported in the literature i.e. 10 pg/mL [55] or 12 pg/mL [56] using ELISA test, demonstrating the robustness of the procedure of ELISA technique in the case of serum matrix.

As an alternative approach to the established ELISA test, in the view of developing a rapid and cost-effective method, Chiang et al. proposed a fiber-optic/plasmon resonance-based device for IL-1β detection in synovial fluid [41]. A microfluidic pathway was used as a sample inlet and outlet, while antibody anti-IL-1β was bound onto the surface of AuNPs, self-assembled on the naked portion of the optical fiber (Fig. 3C). The subsequent introduction and binding of the target analyte with the antibody anti-IL-1β is transduced in an increased refractive index of the medium surrounding the AuNPs. Therefore, an increase in plasmon absorbance of the AuNPs is recorded.

By measuring IL-1β in standard solution a linear behavior within the concentration range of 0.050–10 ng/mL was obtained, with a limit of detection of 21 pg/mL. Finally, the performances of the developed biosensor were compared with the ELISA test for the analyses of 13 synovial fluid samples, obtaining a correlation coefficient equal to 0.985, highly shortening the detection time (i.e., <10 min).

Using the same configuration designed for CRP detection (section 3.1), Borse et al. monitored the presence of IL-6 in serum by a lateral flow immunoassay [32]. In this case, an antibody anti-IL-6 was immobilized on the staphylococcal protein A conjugated with CdTe quantum dots and cast onto the nitrocellulose test strips. The produced fluorescence signal revealed by the portable fluorescence strip reader (PorFloR™), gave a linearity in the range of 1–1000 pg/mL. Finally, the results obtained from spiking serum samples with different IL-6 concentrations agreed with the standard ELISA technique results, obtaining a satisfying correlation coefficient equal to 0.999.

3.3. Lactate

D-lactate, as well as its L-lactate isomer, is mainly produced in the human body by different bacterial species present in the gastrointestinal tract, as well as bacteria during infection states. While L-lactate is readily metabolized in the liver and kidney, with consequent very low levels in the blood, high levels of D-lactate are specifically connected to the presence of gastrointestinal pathologies or infections caused by bacterial species [57,58].

To prove the efficacy of this innovative analyte for PJI diagnosis, two important studies were reported by Renz research group for D-Lactate monitoring in synovial fluids, using a commercial enzyme-based spectrophotometric kit. In the first study [59], 148 clinical synovial fluid samples were analyzed applying PJI criteria stated by the EBJIS [13], obtaining a sensitivity of 86 % and a specificity of 82 %. In the second study, a larger prospective cohort was analyzed, i.e., 224 patients, selecting the PJI criteria stated by the Musculoskeletal Infection Society (MSIS) and the institutional PJI criteria [60]. Using an optimized threshold value of 1.3 mmol/L, sensitivity equal to 94 % and 93 %, with specificity of 78 % and 89 % were obtained using the MSIS and PJI criteria, respectively. Furthermore, the threshold value of 1.3 mmol/L reported in Ref. [60], was found in close agreement with the one reported in Ref. [59], which was equal to 1.263 mmol/L.

More recently Chen et al. compared the D-Lactate levels in serum and synovial fluid from 26 patients after total knee arthroplasty, by ELISA test [61]. The obtained results showed sensitivity of 88 %, and 96 %, and low specificity of 73 % and 68 %, in serum and synovial samples, respectively. As highlighted by the low specificities, the interference of various compounds, such as erythrocytes or other proteins, which absorb a similar wavelength, enhances the false positive ratio.

In summary, D-lactate detection in synovial and serum fluids showed good performance and a promising role in PJI diagnosis. However, confirmatory analyses of synovial fluid biomarkers are still needed to enhance the specificity.

3.4. pH

The value of pH decreases in the presence of infections is well reported in the literature [62], indeed the change in the growth medium's pH is used in classic microbiology to identify bacterial species in vitro [63]. In the case of PJI diagnosis, the decrease of pH is due to the presence of bacteria in the peri-implant surface, as reported by Dong et al. which demonstrated the suitability of pH as a biomarker, finding a pH as low as pH 5.5 around the peri-implant surface during bacterial infection [64].

Following this concept, Fiore et al. [42] developed a miniaturized potentiometric pH sensor based on a screen-printed electrode modified with the H^+ iridium-oxide sensitive layer for the detection of pH of whole blood sampled during orthopedic surgery. pH measurements were carried out using a Bluetooth-assisted device using only 100 μL of blood sample and furnishing the results in less than 1 min (Fig. 3D). The authors highlighted that in the presence of infections (confirmed by microbiological analyses) the blood pH value decreased down to 7.0.

4. From laboratory-setup methods to point-of-care devices: the roadmap in PJI diagnosis

The well-established analytical systems based on ELISA and

Table 2
Main features of laboratory-based analytical systems for PJI biomarkers detection.

Biomarker	Analytical technique	Sensing element	Signal readout instrumentation	LOD	Linear range	Matrix	Ref
CRP	Immunoturbidimetric assay	Antibody	Synchron LX (Beckman Coulter, Inc)	5 mg/L	5–200 mg/L	Synovial fluid (27) Serum (28)	[27, 28]
	ELISA (GenWay Biotech)	Antibody	–	—[a]	—[a]	Synovial Fluid	[28]
	Multiplex ELISA (RulesBased Medicine's Human Inflammation MAP)	Antibody	–	—[a]	—[a]	Synovial Fluid	[28]
	ELISA (Human CRP Quantikine ELISA Kit)	Antibody	–	—[a]	—[a]	Serum and Synovial Fluid	[54]
D-dimer	Immunoturbidimetric assay	Antibody	STA-R analyzer (Stago diagnpstica)	0.27 μg/mL	0.27–20 μg/mL	Serum (23) Plasma (24)	[23, 24]
		Antibody	Sysmex CS-5100 System (Sysmex)	—[a]	—[a]	Plasma	[26]
Alpha-defensin	ELISA	Antibody	—[b]	—[b]	—[b]	Synovial Fluid	[29]
Calprotectin	ELISA	Antibody	–	—[a]	—[a]	Synovial Fluid	[44]
	ELISA (Calprest® NG, Eurospital)	Antibody	–	—[c]	—[c]	Synovial Fluid	[46, 47]
Interleukin	ELISA	Antibody	Multiplex bead-based Luminex 100	—[a]	—[a]	Serum	[52]
	ELISA (ML096281, Shanghai Enzyme-linked Biotechnology)	Antibody	–	–	–	Serum	[53]
	ELISA (Human IL-6 QuantiGlo ELISA Kit, RD)	Antibody	–	0.35 pg/mL	0.48–1500 pg/mL	Serum and Synovial Fluid	[54]
Lactate	Spectrophotometric test (D-lactam Kit; VL-Diagnostics)	Enzyme-based test	Microplate Absorbance Reader (DYNEX Technologies MRX)	–	–	Synovial Fluid	[59, 60]
	ELISA (Kit from Shuang Ying Biological Technology Co., Ltd.,)	Antibody	–	<0.05 mmol/L	—[a]	Synovial Fluid and blood	[61]

ELISA: Enzyme-linked immunosorbent assay.
[a] Parameters not specified in the analytical kit.
[b] It is a meta-analysis of 42 articles.
[c] Only provided in mg per g of feces sample.

Table 3
Main features of commercially available point-of-care devices for PJI biomarkers detection.

Biomarker	Analytical technique	Sensing element	Signal readout instrumentation	LOD	Linear range	Matrix	Ref
Alpha-defensin	LFIA (Synovasure™, Zimmer Biomet)	Antibody	Optical signal	Qualitative test		Synovial Fluid	[29]
	LFIA (Zimmer Biomet)	Antibody	Optical signal	Qualitative test		Synovial Fluid	[30]
Leukocyte esterase	Colorimetric test (LE Combur strip test, Roche Diagnostics)[a]	Enzymatic reaction, followed by diazonium salt reaction giving a purple color	Optical signal	Qualitative test		Synovial Fluid	[30]
	Colorimetric test (LE Chemstrip, Roche Diagnostics)[a]	Enzymatic reaction, followed by diazonium salt reaction giving a purple color	Optical signal	Qualitative test		Synovial Fluid	[31]
Calprotectin	LFIA (Quantum blue®, BÜHLMANN laboratories)[b]	Antibody	Quantum Blue® reader	—[c]	—[c]	Synovial Fluid	[45]
	LFIA (Calfast® NeXT, Eurospital)[b]	Antibody	CalFast® reader (Eurospital)	–	50–300 mg/L	Synovial Fluid	[46]
	LFIA (Lyfstone AS®)	Antibody	Smartphone with dedicated App	2.99 mg/L	14–300 mg/L	Synovial Fluid	[48–50]

LFIA: Lateral flow immunoassay, LE: Leukocyte esterase: POC: Point-of-care.
[a] Specifically developed for urine analysis.
[b] Specifically developed for fecal analysis.
[c] Only provided in mg per g of feces sample.

immunoturbidimetric assay are used to detect CRP, alpha-defensin, D-dimer, calprotectin, and interleukin in synovial fluid and in serum samples, while enzymatic assay is selected for lactate quantification (Table 2). These methods, characterized by high accuracy, require signal readout instrumentation based on laboratory setup.

For developing point-of-care devices, lateral flow and colorimetric detection have been widely exploited for the fabrication of diagnostic analytical tools for market entry. For this reason, Zimmer Biomet, Roche, and Lyfstone companies produced point-of-care devices for the detection of alpha-defensin, leukocyte esterase, and calprotectin in synovial fluid based on lateral flow fluidics and colorimetric detection (Table 3). Additionally [45,46], extended the use of lateral flow devices developed for the detection of calprotectin in fecal samples to synovial fluid matrix, demonstrating the robustness of this type of devices.

The analytical tools for alpha-defensin and leukocyte esterase are characterized by qualitative detection, while the calprotectin quantitative detection identified different ranges for giving low, moderate, and high risk of infection [50].

In the last decade, the scientific community have developed several point-of-care devices harnessing advanced systems based on microfluidics, nanomaterials, and smart electronics (Table 4) to deliver highly sensitive and robust diagnostic tools for improved PJI diagnosis, demonstrating the utility of using advanced technologies to face the reliable PJI diagnosis issue.

Table 4
Analytical features of sensors reported in the literature for PJI biomarkers detection.

Biomarker	Analytical technique	*Signal readout instrumentation*	Portability	LOD	Linear range	Interference studies	Matrix	Accuracy	Storage Stability	Advantages	Disadvantages	Ref.
CRP	LFIA, Fluorescence	PorFloR™ custom-fabricated fuorescence reader	YES	0.3 μg/mL	1–300 μg/mL	–	Serum	-RSD: 0.22–6.67 % [a] R^2: 0.9978 [b]	9.68 C V % up to 90 days	Assay time	Price for quantum dots	[32]
	LFIA, Colorimetric	CCD camera and ImageJ software	YES	–	15.5–310 ng/mL	–	Synovial Fluid and serum	-RSD in diluted Synovial Fluid: 34–193 % [a] -RSD in diluted Serum: 139–175 % [a]	–	Quantitative LFIA strip	Low accuracy	[36]
	LFIA, Colorimetric	CCD camera and ImageJ software	YES	–	–	–	Synovial Fluid	R^2: 0.92 [b]	–	Assay time, camera reader, multiple detection analyte	Interferences (External light, blood)	[33]
	Luminescence	FLUOstar Omega optical detection module (BMG LabTech)	NO	1 mg/L	1–100 mg/L	Vs HSA, IgG, IL-6, and PCT	Synovial Fluid	R^2: 0.993 [b]	–	Multiple analyte detection, microfluidic chip for automatic detection	Bulk reader	[34]
Alpha-defensin	LFIA, Colorimetric	CCD camera and ImageJ software	YES	–	–	–	Synovial Fluid	R^2: 0.91 [b]	–	Assay time, Camera reader, multiple detection analyte	Interferences (External light, blood)	[33]
	Luminescence	FLUOstar Omega optical detection module (BMG LabTech)	NO	0.01 mg/L	0.01−50 mg/L	Vs HSA, IgG, IL-6, and PCT	Synovial Fluid	R^2: 0.975 [b]	–	Multiple analyte detection, microfluidic for automatic detection	Bulk reader	[34]
	Fluorescence	FLUOstar Omega optical detection module (BMG LabTech)	NO	0.2 mg/L	0.5−100 mg/L		Synovial Fluid	CV: 2.8–24.1 % [b]	–	Microfluidic chip for automatic detection	Bulk reader	[35]
	Fluorescence	Fluorescent microscope (bx43, Olympus)	NO	0.5 mg/L	Up to 100 mg/L	Vs HSA, IgG, IL-6, and PCT	Synovial Fluid	CV: 13–25 % [b]	–	Microfluidic chip for automatic detection	Bulk reader, Interferences (External light)	[38]
Leukocyte esterase	Colorimetric test (LE Aution Sticks 10 P A, Arkray)	RGB photometric system	YES	–	–	–	Synovial Fluid	–	–	Quantitative LFIA strip	Interferences (External light, blood)	[37]
	Coulometry by glucose test strip	Bench potentiostat (CHI 832 B, CH Instruments)	NO	20 μg/L	up to 750 μg/L	–	Synovial Fluid and urine	–	–	Quantitative test strip, no matrix interference (turbidity, color)	Bulk potentiostat	[39]
	Coulometry by glucose test strip	Commercial glucometer (ACCU-CHEK Aviva Plus, Roche Diabetes Care) and bench potentiostat (CHI 832 B, CH Instruments)	YES	25 μg/L	up to 800 μg/L	–	Synovial Fluid and urine	–	–	Quantitative test strip, no matrix interference (turbidity, color)	–	[40]
Interleukin	LFIA, Fluorescence	PorFloR™ custom-fabricated fluorescence reader	YES	0.9 pg/mL	1–1000 pg/mL	–	Serum	-RSD: 0.53–13.33 % [a] -R^2: 0.9994 [b]	2.65 C V % up to 90 days	Assay time	Price for quantum dots	[32]
	Fiber-optic/ plasmon resonance-based device	Photoreceiver 2001, New Focus	YES	21 pg/mL	0.050–10 ng/mL	Vs HSA	Synovial Fluid	R^2: 0.985 [b]	–	Assay time	–	[41]
pH	Potentiometry using SPE	Portable potentiostat (EmStat3 Blue, PalmSens)	YES	–	pH 3-7	–	Synovial Fluid, granulocyte cell exudate samples	Infection positivity in agreement with microbiological analyses	Up to 30 days	Assay time	–	[42]

CV: coefficient of variation, LFIA: Lateral flow immunoassay, L.E.: Leukocyte esterase, HSA: human serum albumin, POC: Point-of-care, RGB: Red green blue, PCT: procalcitonin, SPE: Screen-printed electrode.
[a] Calculated by recovery studies.
[b] Calculated vs reference method.

Table 5
Main features of implantable devices developed for PJI biomarkers detection.

Biomarker	Analytical technique	Sensing element	Sensor configuration	Signal readout instrumentation	LOD	Linear range	Matrix	Ref
pH	Potentiometry using titanium (Ti–6Al–4V) working electrode	Polyaniline film	Titanium plaque	6-channel high input impedance voltmeter (Lawson Laboratories)	–	pH 5-8	Standard solution with BSA	[65]
pH	Radiographic measurements after hydrogel swelling	Poly (AAc-*co*-*n*-OA) hydrogel film	Sensor attached to an orthopedic plate fixed to a tibia	X-ray imaging instrument	–	pH 4-8	Buffer solution	[66]
pH	Radiographic measurements after hydrogel swelling	Poly (AAc-*co*-*n*-OA) hydrogel film	Sensor attached to the neck of the hip prosthetic implant	X-ray imaging instrument	–	pH 4-8	Bovine synovial fluid	[67]
***E. Coli* Bacteria**	Magnetoelastic immunosensing	Antibodies	Packaged sensor integrated into a recess on a prosthetic knee joint	Resonance frequencies network analyzer (Keysight® E5061B)	–	1–5.9 cP[a]	*E.coli* suspensions	[68]

Poly(AAc-*co*-*n*-OA): acryl acid and n-octyl acrylate polymer.
[a] Viscosity unity: 1 cP = 1 mPa s (1 mPa per second).

5. Implantable devices

High technological development in terms of wireless data transmission, chip miniaturization, and biocompatible materials, is recently contributing to the fabrication of implanted chemical sensors to monitor patients' infection after a periprosthetic implant. Thus, this sensor technology has the potential to allow for i) long-term monitoring of chemical parameters around the periprosthetic implant and eventually infected zone, ii) identification of complications or failures following orthopedic implants.

In the overall scenario of implantable devices, few examples have been reported in the literature related to PJI monitoring using chemical sensors (Table 5).

Tomšík et al. [65] developed a potentiometric pH sensor based on polyaniline as a sensitive layer to H^+ ions. In detail, polyaniline was chemically deposited on a titanium alloy (Ti–6Al–4V), an established implant fabrication material. Interestingly, to protect the polyaniline film from naturally occurring fouling in synovial fluids, a poly (2-methyl-2-oxazoline) film was electrochemically fixed onto the polyaniline/titanium alloy surface. The developed potentiometric sensor is able to detect pH in the range between pH 5 and 8, with a Nernstian slope of −59.6 mV/pH. Finally, synovial fluids from a first group of 18 patients with confirmed joint infection, and a second group of 19 patients without a current infection process were analyzed. In the first case, the average pH of the synovial fluid was 6.95 ± 0.47, while a higher pH of 7.75 ± 0.32 was obtained for non-infected synovial fluid.

A different approach to monitoring the pH variation in infected solution based on the swelling of H^+-sensitive hydrogels has been reported by Arifuzzaman et al. [66] and Wijayaratna et al. [67]. The authors leveraged the gel composition, which is able to swell the whole gel structure (expansion and contraction) at different pH values, and plain radiography for the swelling evaluation.

In detail, Arifuzzaman et al. [66] evaluated the swelling of the hydrogel disk by measuring the position variation of a radiopaque tungsten rod attached to the disk edge (Fig. 4A). Indeed, the hydrogel expands at low pH values and contracts at higher pH. Measurements in standard solution were linear between pH 4 and 8, fulfilling the pH range typically seen in infected and healthy tissues. Radiographic measurements were also performed in bacterial culture and cadaveric tissue with the sensor attached to an orthopedic plate fixed to a tibia.

Wijayaratna et al. [67] exploited polyacrylic acid-based hydrogel, which expands at high pH and contracts at low pH, thereby moving a radiodense tantalum bead embedded in the hydrogel (Fig. 4B). The plain radiograph shows the tantalum bead position change relatively to a tungsten wire, marking the zero position at pH 7. The sensor showed a linear response in the physiologically relevant pH range of pH 4-8. Finally, the sensor was attached to an explanted prosthetic hip, and the pH response was determined in bovine synovial fluid solutions, by five observers. The results obtained were in good agreement among the values recorded by the five observers and consistent with values recorded by the reference method, showing good accuracy of the developed sensor.

However, despite showing potential use for implanted sensor development, the proposed device suffers from important drawbacks. Indeed, plain radiography requires an X-ray source, which limits measurements to a low number of time points, not enabling continuous measurement in live patients. Furthermore, the visual scale needed to measure the hydrogel position limits the objectivity of the readout system. More recently, Jiang et al. [68] focalized the study on bacterial detection, by functionalizing a metal alloy sensor surface with antibodies targeting specific types of bacteria. The sensor comprises a $Fe_{45}Ni_{45}Mo_7B_3$ metal alloy with a Cr/Au layer for antibody immobilization and was mounted in a biocompatible package integrated into a cavity on a prosthetic knee joint (Fig. 4C). The transduction mechanism is based on the magnetoelastic principle, enabling the wireless interrogation of the sensor, without the need for an antenna or battery. Briefly, a transmitting coil generates a magnetic field, giving a magnetic flux with a resonance frequency, which varies on the boundary conditions of the sensor, such as mass or fluid medium. When the antibody captures the target bacteria, namely *E. coli*, a change in frequency is recorded by a network analyzer, able to obtain the resonance frequencies of the sensors. The feasibility of the sensor for in vitro bacterial detection was assessed in a different fluid medium viscosity, i.e., *E. coli* suspensions with different viscosities, ranging between 1 and 5.9 cP.

6. Conclusions and perspectives

Nowadays the diagnosis of PJI remains a relevant challenge to address, considering that PJI represents one of the most devastating complications of orthopedic surgery, leading to the failure of the prosthetic implant and, in the most serious cases, causing a systemic infection that can risk the life of the patient. Furthermore, PJI is considered to be one of the costliest infectious diseases to treat, considering that its treatment requires at least one surgery, prolonged hospitalization, rehabilitation care, prolonged antibiotic therapy, and extended absence from work in working-age patients. The diagnosis of PJI remains a big challenge as highlighted by Moriarty et al. [4] who reported that the correct diagnosis with an innovative test would be a major breakthrough to face PJI diagnosis, considering the high number of culture-negative infections. The authors highlighted that there is an urgent need for a diagnostic device able to provide accurate and rapid diagnosis of PJI. Indeed, the classical approaches for PJI diagnosis have several drawbacks.

- The diagnosis based on symptoms such as pain, swelling, and fever are non-specific;

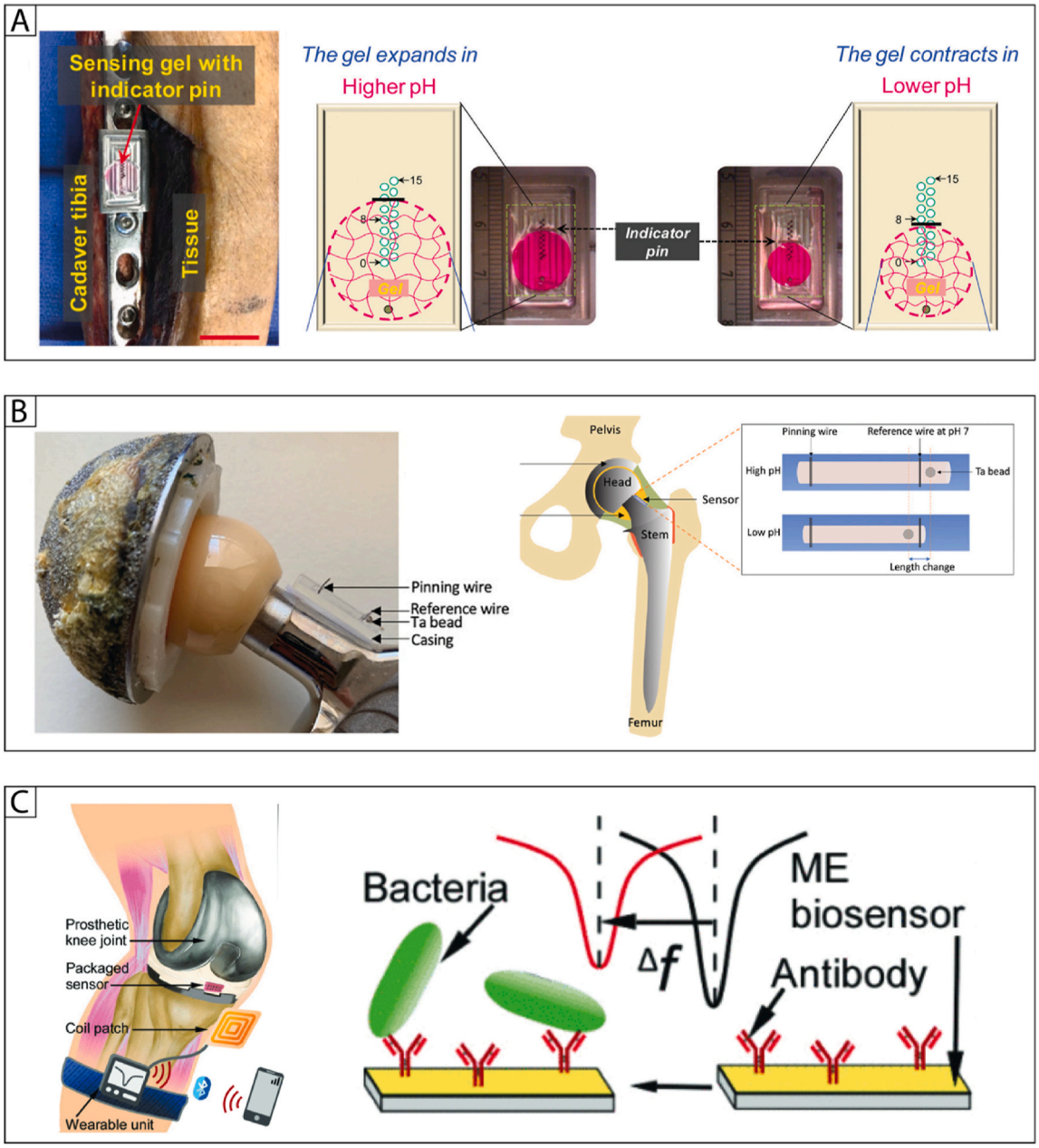

Fig. 4. Implantable periprosthetic joint infection diagnostic tools. A) Radiographic detection of hydrogel swelling for pH detection in cadaveric tissue with the sensor attached to an orthopedic plate fixed to a tibia [66]. B) radiographic detection of hydrogel swelling for pH detection with the sensor attached to an explanted bovine prosthetic hip [67]. C) Magnetoelastic transduction mechanism-based in vitro bacterial detection with the sensor implanted into a cavity on a prosthetic knee joint [68].

- Radiography is not suitable at the early stage because the biofilms are often localized on inaccessible regions of implant surfaces;
- Magnetic resonance imaging and computed tomography can detect bone resorption and sinus tracts, but they are not sensitive at the early stage of infection;
- The analysis of specimens taken from periprosthetic tissue is invasive and does not provide accurate information for the restricted area sampled, reason for several criteria including the quantification of selected biomarkers in serum and synovial fluid have been established to help the prompt individuation of PJI.

To face the issues of PJI diagnosis, criteria for PJI diagnosis were established in 2013, updated in 2018, and further updated in 2021, demonstrating up to now the need for the selection of proper biomarkers (well-established, emerging, and others [17]) together with accurate and sensitive diagnostic tools. In the last decade, there has been a growing interest in point-of-care devices, starting from the demonstration of their utility with colorimetric lateral flow devices to the development of smart (bio)sensors using advanced technologies for well-established and emerging biomarkers (Fig. 1A). However, the small number of articles highlighted that is a field that needs to be further investigated to supply point-of-care devices with high Technology Readiness Level (TRL) values able to reach the market and change the field of PJI diagnosis, considering that to reach the market the medical devices need to accomplish the analytical required features namely robustness and accuracy with their assessment in the clinical studies.

The further improvement of point-of-care devices is related to the implantable sensors. In this regard, Karipott et al. [69] shed light on the important role of implantable wireless sensors in orthopedic care to

understand the progression of PJI and the effectiveness of treatments. The advancements in sensors, wireless communication, power management, microelectronics, and other technologies will boost the design of effective safer implantable wireless sensors.

These concepts further demonstrated that the sensing discipline can have a large impact on PJI diagnosis, monitoring, and management and the recently developed sensitive point-of-care devices can be only a starting point.

CRediT authorship contribution statement

Vincenzo Mazzaracchio: Writing – original draft. **Raffaele Vitiello:** Writing – review & editing. **Giulio Maccauro:** Funding acquisition, Writing – review & editing. **Fabiana Arduini:** Conceptualization, Funding acquisition, Writing – review & editing, Writing – original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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