Next Generation Sequencing for the Detection of Pathogens in Hip Surgery: Experience and Diagnostic Feasibility in a Tertiary Care Center in Argentina

Carlos M. Lucero, Agustín García-Mansilla, Agustín Albani Forneris, Fernando Díaz Dilernia, Pablo Slullitel, Gerardo Zanotti, Fernando Comba, Francisco Piccaluga, Martín Buttaro

Hip Center "Sir John Charnley", Institute of Orthopedics and Traumatology "Prof. Dr Carlos E. Ottolenghi", Hospital Italiano de Buenos Aires, Autonomous City of Buenos Aires, Argentina

ABSTRACT

Introduction: Early diagnosis of a periprosthetic joint infection (PJI) and identification of the pathogen are paramount. Next-generation sequencing (NGS) can identify the nucleic acids in a given germ in a short period. To our knowledge, there are no reports of its use in the management of PJI in South America. Our objective was to demonstrate the diagnostic feasibility of the NGS technique on the samples obtained from a series of patients operated on in Buenos Aires, Argentina. **Materials and Methods:** A prospective series of 20 patients undergoing septic and aseptic hip revision surgery from December 2019 to March 2020 was analyzed. Intraoperative samples of synovial fluid, deep tissue, and intramedullary canal were obtained and sent to the NexGen Microgen laboratory (Texas, USA) for analysis. **Results:** Seventeen patients were finally eligible to present a sample suitable for analysis. In 100% of the samples, NGS results were obtained within 72 hours of surgery. In one case, the NGS result reported a germ different from the one identified in the postoperative soft tissue cultures, allowing antibiotic therapy to be corrected. In another case, NGS identified *Parabacteroides gordonii* in aseptic revision surgery. In another patient, the NGS identified *Morganella morganii*, in which conventional postoperative cultures were negative in single-stage revision surgery. **Conclusion:** In this study, we demonstrated the diagnostic feasibility of NGS, obtaining results within 72 hours immediately after surgery for pathogenic organisms in patients with PJI and negative cultures.

Keywords: Periprosthetic joint infection; next generation sequencing; revision surgery; hip arthroplasty.

Level of Evidence: IV

Secuenciación de próxima generación para la detección de patógenos en cirugía de cadera: experiencia y viabilidad diagnóstica en un centro de atención terciaria de la Argentina

RESUMEN

Introducción: El diagnóstico rápido y definitivo con identificación del patógeno es fundamental cuando hay una infección periprotésica. La secuenciación de próxima generación permite identificar el ADN en un germen determinado en un período de tiempo corto. Hasta donde sabemos, no hay reportes sobre su empleo para el manejo de la infección periprotésica en Sudamérica. Nuestro objetivo fue demostrar la viabilidad diagnóstica de las muestras obtenidas de una serie de pacientes operados en Buenos Aires, Argentina, y analizadas con la técnica de secuenciación de próxima generación. **Materiales y Métodos:** Se analizó a una serie prospectiva de 20 pacientes sometidos a cirugía de revisión séptica y aséptica de cadera desde diciembre de 2019 hasta marzo de 2020. Se obtuvieron muestras intraoperatorias de líquido sinovial, tejido profundo y canal endomedular, que fueron enviadas para su análisis al laboratorio NexGen Microgen (Texas, EE.UU.). **Resultados:** Se seleccionaron 17 pacientes, porque una muestra era apta para el análisis. Todos los resultados se recibieron dentro de las 72 h de la cirugía. En un caso, el resultado de la secuenciación de próxima generación informó un germen distinto del identificado en los cultivos posoperatorios de partes blandas, lo que permitió corregir la antibioticoterapia. En otro, esta técnica identificó *Parabacteroides gordonii* en una revisión aséptica. En otro paciente, identificó *Morganella morganii*, a partir de cultivos negativos en una revisión en un tiempo. **Conclusión:** Se demostró la viabilidad diagnóstica con la secuenciación de próxima generación, se pueden obtener resultados dentro de las 72 h posteriores a la cirugía de microorganismos patógenos en pacientes con infección periprotésica y cultivos negativos.

Palabras clave: Infección periprotésica; secuenciación de próxima generación; cirugía de revisión; artroplastia de cadera.

Nivel de Evidencia: IV

Received on May 4th, 2022. Accepted after evaluation on July 16th, 2022 • Dr. CARLOS M. LUCERO • cm.lucero@hotmail.com • https://orcid.org/0000-0003-1325-7027

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INTRODUCTION

Periprosthetic joint infection (PJI) is a rare but devastating complication that is associated with a higher rate of morbidity and mortality.[1,2] Managing this scenario is challenging and costly, and requires particular expertise to achieve an optimal result.[3,4]

Making a quick and definitive diagnosis with the identification of the causal microorganism is fundamental for the management of a PJI,[5-7] since not identifying the infecting germ leads to the administration of an empirical antimicrobial treatment, with the possibility of not covering the true pathogen. On the other hand, a negative culture has been associated with a 4.5 times higher risk of reinfection than a positive one.[8] There are various bacteriological culture techniques, such as polymerase chain reaction, to improve diagnostic accuracy. However, polymerase chain reaction has a limited sensitivity ranging from 50% to 81.6%,[9,10] and, on the other hand, it is ineffective in identifying fungal or polymicrobial infections, and differentiating contaminants from the true infectious organism.[10.11]

Next-generation sequencing (NGS) is a novel and cost-effective technique that can identify all nucleic acids in a given germ in a short period of time. It is capable of sequencing all the DNA present in a sample and provides more complete information on the microbial profile,[12] which allows efficient identification of the genomes of bacteria and fungi. Tarabichi et al.[13] demonstrated the usefulness of this technique by identifying potential pathogens in 81.9% of PJI cases with a negative culture.

The use of this technology is possible in our country; however, carrying out the sequencing to obtain a specific germ is not enough, a database is also needed with which to compare the sequences obtained, and thus determine the microorganism that has such sequencing. Therefore, the larger the database, the more likely it is to obtain a specific and reliable match. In this sense, the NexGen Microgen laboratory (Texas, USA) has the program with the largest known database in the world, including DNA sequences obtained from lunar rocks. The main challenge researchers face is limited resources. As a result, genomic tools, specifically genome sequencing technologies, are not widely available because of the operational cost to implement them, shipping costs, customs costs, and the profit margin for local companies, not to mention the distance to the target laboratory. These factors could delay the transfer and analysis of the sample and, in this way, alter its quality and results.

To our knowledge, there are no reports on the use of this technique in the management of PPIs in South America. The objective of this study was to prospectively demonstrate the viability of samples obtained from a cohort of patients operated on in a tertiary care hospital in Argentina, which were analyzed with the NGS technique in the NexGen Microgen laboratory (Texas, USA). Secondly, to evaluate the role of NGS in detecting microorganisms in a cohort of patients undergoing septic and aseptic revision hip surgeries.

MATERIALS AND METHODS

Patients

After obtaining approval from the Ethics Committee of our institution, we prospectively analyzed a series of 20 patients who agreed to participate in the study and had undergone total hip arthroplasty revision surgery between December 2019 and March 2020. Patients diagnosed with PJI and aseptic loosening, as defined by the Musculoskeletal Infection Society (MSIS) criteria, were included.[14]

Preoperative evaluation

All patients were evaluated before surgery according to institutional protocols, which included blood tests to determine glomerular filtration rate (GFR), C-reactive protein (CRP), and D-dimer. Preoperative antibiotics were suspended two weeks before the index surgical procedure until the collection of samples for culture, pathology analysis, and NGS.

All patients had access to the results of the preoperative blood tests used for diagnosis; however, not all had a biopsy and PCR of preoperative biological fluid. Since 2014, the Hip Department of our institution has indicated to evaluate revisions with clinical suspicion of infection by intraoperative synovial PCR, and a value >9.5mg/l is considered positive.[15]

During the study period, two-stage surgery was indicated in the following cases: 1) upon confirmation of chronic PJI according to the MSIS[14] criteria; and 2) to functionally active patients, with independent or minimally aided walking (index of instrumented activity of daily living ≤7).[16] Similarly, single-stage surgery was indicated in the following cases: 1) those with confirmation of chronic PJI according to the MSIS criteria,[14] but without fistula or active wound drainage, 2) patients with low functional demand (index of instrumented activity of daily living >7)[16] and 3) acetabular bone stock with a defect lower than grade 3 of the Paprosky classification[17] and femoral bone stock with a defect lower than or equal to grade 3B of that classification.[17,18]

Intraoperative sample collection

Patients were placed in the lateral decubitus position, and a posterolateral hip approach was performed in a laminar flow operating room. During anesthetic induction, each patient received a dose of antibiotic adapted to them. Since 2011, we have administered a dose of 1,000 mg of tranexamic acid, intravenously, during anesthetic induction and an additional 1,000 mg during closure, in all surgeries.[19] The revision surgeries were performed by four specialist hip surgeons from our institution.

Synovial fluid, deep tissue, and intramedullary canal samples were taken from all patients at the time of surgery. Synovial fluid was obtained sterile, using an 18-gauge needle before arthrotomy. Lastly, the femoral acetabulum and intramedullary canal were swabbed.

All samples were quickly collected in sterile containers and sent for study by private courier. Deep tissue samples were also sent to the institutional laboratory for routine cultures, including cultures of aerobic and anaerobic bacteria, fungi, and acid-fast bacilli. Likewise, samples were sent to pathology for analysis by freezing and PCR of intraoperative synovial fluid was requested.

Next generation sequencing

DNA extraction

DNA extraction is performed on submitted samples with the QIAamp DNA Mini Kit column-based extraction kit (Qiagen GmbH, Hilden, Germany). The samples are treated in a differential way to adapt them to the DNA extraction protocol:

- Synovial fluid: the fluid is centrifuged and 200 µl are extracted as starting material for the extraction.
- Deep soft tissue: the tissue is cut with a sterile scalpel blade into pieces of approximately 1 mm^3 and up to 20 mm^3 of representative material is mixed with a phosphate-buffered saline solution until reaching a final volume of 200 µl and continuing with the extraction.
- Intramedullary canal material: it is suspended in phosphate-buffered saline to a final volume of 200 µl and then extracted.

Once the sample has been adapted to the protocol, DNA extraction is performed following the manufacturer's instructions. The elution volume is 50 µl. The samples are placed in 1.5 ml tubes and stored in a refrigerator at -20°C until use.

Shipment of samples

From the DNA of each of the samples, an aliquot of 15 µl is placed in a 0.5 ml tube and labeled according to its identifier. The DNA sample is sent by FedEx® in a container to the NexGen Microgen laboratory (Texas, USA).

NGS Study

The NexGen Microgen laboratory carries out NGS studies by amplification of the 16S rRNA gene in the samples sent to detect pathogens of bacterial origin.

Bioinformatic analysis and reporting of results

The NexGen Microgen laboratory analyzes the data obtained according to its own protocol and sends the results to our hospital by email within 72 hours to complete the database.

Antibiotic treatment

After surgery, when a case was considered to be septic, a course of intravenous antibiotic therapy was administered for six weeks, according to the criteria of the surgeon and the Infectious Disease Service of our institution. When the typing of the germ and its sensitivity were adequate and the selected antibiotic reached adequate bioavailability by the oral route, this route of administration was chosen. Infection control was carried out 15 days, a month, and six weeks after the surgical procedure, and clinical findings were controlled, such as the state of the wound, the presence of pain, and the results of blood tests (GFR, CRP).

RESULTS

NGS was performed in 20 cases, and 17 were selected, because they had a suitable sample for analysis. The first three cases were discarded because permission from the Ministry of Health to ship the samples abroad could not be obtained in time. Although the samples were stored in the refrigerator below -80°C, after 3-5 days, they were considered to have lost their quality for testing due to denaturation of nucleic acids.

The series consisted of 17 patients, 64.70% (11 patients) were men and 35.30% (6 patients) were women. Nine cases (52.95%) corresponded to the left hip and eight (47.05), to the right. The average age was 68 years (range 37-86). Before surgery, 10 revisions (58.83%) were interpreted as aseptic and the remaining seven (41.17%) as septic. The surgeries were: single-stage revisions (9 cases; 52.94%) and two-stage revisions (7 cases; 41.17%); in four of them (57.14%), it was the first surgical stage (spacer placement) and, in three (42.86%), the second stage (reimplantation). Lastly, the remaining surgery (5.89%) was debridement with sampling and implant retention. Table 1 summarizes the demographic data of the series.

Table 1. Patient data.

Case	Age	Sex	Side	Septic/Aseptic	Surgical stage
1	77	M	R	Septic	2nd. spacer
2	73	M	L	Aseptic	1°
3	86	F	L	Aseptic	1°
4	77	F	L	Aseptic	1°
5	37	M	L	Septic	2nd. Reimplantation
6	77	M	R	Septic	2nd. Reimplantation
7	72	F	R	Aseptic	1°
8	73	F	L	Septic	Debridement
9	61	M	R	Aseptic	2nd. spacer
10	43	M	R	Aseptic	1°
11	74	F	L	Septic	2nd. Reimplantation
12	54	M	L	Aseptic	1°
13	67	M	R	Septic	2nd. spacer
14	81	M	L	Aseptic	1°
15	65	M	R	Aseptic	1°
16	67	M	R	Aseptic	1°
17	78	F	L	Septic	2nd. spacer

M = male, F = female, L = left, R = right.

Positive preoperative cultures were obtained in seven (41.17%) patients (cases 1, 2, 5, 8, 11, 13, and 17). The predominant germ was methicillin-sensitive *Staphylococcus aureus* (MSSA) (3 cases; 42.85%), followed by methicillin-resistant *S. aureus* (MRSA) (2 cases, 28.57%) and *Propionibacterium acnes* and *Escherichia coli*, one case each (14.28%). Only four (57.14%) of the seven patients with positive preoperative cultures had an acute inflammatory process in the frozen pathology analysis of revision surgery, the remaining three cases (42.85%) were non-inflammatory processes.

The preoperative infectious staging was different according to each particular case and, due to the heterogeneity of the sample, the GFR, CRP and D-dimer values obtained in the preoperative period, as well as the preoperative cultures and the anatomopathological analysis by freezing, were grouped in Table 2.

Table 2. Preoperative infectious analysis

Case	Preoperative GFR	preoperative CRP	Preoperative D-dimer	Preoperative culture	Pathology anatomy
1	74	89	-	*Staphylococcus epidermidis*	AIR
2	67	4	-	MSSA	NAIA
3	20	1	-	-	Metal debris
4	51	2	5821	-	NAIA
5	60	18	-	MRSA	NAIA
6	34	9	4201	-	NAIA
7	10	5	-	-	NAIA
8	102	55	-	MRSA	NAIA
9	21	4	-	-	AIR
10	11	5	-	-	NAIA
11	57	3	-	MSSA	AIR
12	13	8	-	-	NAIA
13	67	59	-	*Propionibacterium acnes*	AIR
14	14	7	-	-	NAIA
15	55	45	-	-	AIR
16	10	1	506	-	NAIA
17	65	16	-	*Escherichia coli*	AIR

GFR = glomerular filtration rate, CRP = C-reactive protein, MRSA = Methicillin-resistant Staphylococcus aureus , MSSA = Methicillin-sensitive Staphylococcus aureus , AIR = acute inflammatory response, NAIA = no acute inflammatory activity

Regarding postoperative infectious staging, the PCR analyses of synovial fluid and synovial fluid and soft tissue cultures of all cases were recorded and their results are shown in Table 3.

Table 3. Postoperative infectious analysis

Case	Synovial fluid CRP	Synovial fluid culture	Soft tissue culture
1	12	S. epidermidis	S. epidermidis/P. aeruginosa
2	0.2	Negative	Negative
3	0.6	Negative	Negative
4	0.8	Negative	Negative
5	13	Negative	MRSA
6	4.5	Negative	*S. haemolyticus*
7	0.2	Negative	Negative
8	24	*E. faecalis*	*E. faecalis*
9	0.2	Negative	Negative
10	0.5	Negative	Negative
11	0.4	Negative	Negative
12	0.6	Negative	Negative
13	28.5	Negative	Negative
14	0.3	Negative	Negative
15	11.6	Negative	Negative
16	0.4	Negative	Negative
17	4.1	Negative	E.coli

CRP = C-reactive protein, MRSA = Methicillin-resistant Staphylococcus aureus , MSSA = Methicillin-sensitive Staphylococcus aureus.

NGS results

Results of all samples submitted for NGS were received within 72 h post-surgery (Table 4). Nine (53.94%) samples were negative for genetic material corresponding to known bacterial sequences. In one of them (case 9), two-stage surgery was decided due to intraoperative findings suggestive of infection and the results of the pathology analysis that reported an acute inflammatory process. Said patient evolved favorably and, until the last follow-up, had been implanted for 22 months, with no failures or reoperations. In case 11, also with a negative NGS result, the second revision stage was performed without identification of the germ by postoperative culture, but with suspicion of infection due to the pathology anatomy analysis that suggested an acute inflammatory process and positive preoperative cultures for MSSA.

In case 5, the result of the NGS reported a different germ (*Malassezia sympodialis*) from the one identified in the postoperative soft tissue cultures (MRSA). This allowed the case to be correctly interpreted and the postoperative suppressive antibiotic therapy to be adjusted.

In patient 12, NGS allowed the identification of *Parabacteroides gordonii* sensitive to clindamycin and metronidazole when the diagnostic interpretation had been aseptic and the results of the pathology analysis had reported no acute inflammatory changes and negative postoperative cultures, so it was considered a false positive diagnosis by the surgeon and corroborated by the laboratory when discussing the findings.

Likewise, the application of NGS was decisive in patient 15. After a single-stage revision with negative pre- and postoperative cultures and frozen-section pathology analyses suggestive of an acute inflammatory process, it was possible to isolate *Staphylococcus epidermidis* DNA sequences in soft tissue samples, although not in the synovial sample. Although it could be interpreted as a false positive due to contamination, it was decided to administer adjuvant antibiotic treatment.

Table 4. Next Generation Sequencing (NGS) Results

Soft tissue NGS	Synovial fluid NGS	Antibiotic therapy
P.aeruginosa/S. epidermis	*P.aeruginosa/S. epidermis*	Ceftolozane/tazobactam + vancomycin
Negative	Negative	Levofloxacin 750 mg + minocycline 100 mg
Negative	Negative	No
Negative	Negative	No
Malassezia sympodialis	*Malassezia sympodialis*	Vancomycin + ertapenem 1 g Prolonged treatment
Negative	Negative	Ceftolozane/tazobactam + vancomycin
Negative	Negative	No
E. faecalis	*E. faecalis*	Vancomycin 1 gc/12 h
Negative	Negative	No
Negative	Negative	No
Negative	Negative	Levofloxacin 750 mg/day
Negative	*Parabacteroides gordonii*	No
Negative	*Cutibacterium acnes*	Vancomycin 1 gc/12 h/ceftriaxone 2 g/day
Negative	Negative	No
S. epidermidis	Negative	Vancomycin/ciprofloxacin
Morganella morganii	Negative	No
*Corynebacterium*sp 54%; *Corynebacterium mucifaciens* 18%; *E. coli* 12%; *Cutibacterium acnes* 5%; *Lactobacillus crispatus* 4%; *Bradyrhizobium yuanmingense* 2%; *Corynebacterium tuberculostearicum* 2%	*Corynebacterium* sp 54%; *Corynebacterium mucifaciens* 18%; *E. coli* 12%; *Cutibacterium acnes* 5%; *Lactobacillus crispatus* 4%; *Bradyrhizobium yuanmingense* 2%; *Corynebacterium tuberculostearicum* 2%	Vancomycin 2 g / Ciprofloxacin 750 mg suppressive treatment

Similarly, in patient 16, NGS identified *Morganella morganii* in soft tissues, failing to identify organisms in synovial fluid, and acute-phase reactants did not suggest infection and cultures were negative in a patient who underwent a single-stage revision.

Finally, the NGS results also changed the indications in case 17. The revision was planned at one stage due to a suspected multidrug-sensitive *Escherichia coli* infection isolated before surgery. Then, during the operation and by decision of the surgeon, a two-stage revision was chosen, a spacer was made and samples were taken, which were positive for *Escherichia coli* sensitive to multiple drugs. However, the results of the NGS made it necessary to correct the antibiotic therapy and to administer a post-implantation suppressive treatment, since it identified sequences of *Corynebacterium* sp (54%), *Corynebacterium mucifaciens* (18%), *Escherichia coli* (12%), *Cutibacterium acnes* (5%), *Lactobacillus crispatus* (4%), *Bradyrhizobium yuanmingense* (2%), and *Corynebacterium tuberculostearicum* (2%).

DISCUSSION

This study has shown that the use of NGS is feasible in Argentina for the diagnosis of an infection associated with a hip prosthesis, since the distance greater than 8000 km that separates the medical center from the molecular analysis laboratory was not an impediment to analyze, without inconvenience, all the samples sent, and obtaining a result in less than 72 hours. It is important to note that the samples were sent by private courier, without the requirement of specific transport measures that could hinder logistics. Furthermore, in recent years, the costs of these molecular analysis technologies have decreased and they have become relatively affordable diagnostic tools.[20] The NGS technique is already used in our country in various medical specialties, such as in the diagnosis of infertility[20,21] or in the differential diagnosis of specific types of muscular dystrophy.[22] The implementation of these molecular techniques for the diagnosis of a periarticular infection both in our country and in the rest of Latin America is novel and we did not find publications that report on their use. Perhaps their use is limited due to the lack of suitable local laboratories with extensive molecular databases that allow the correct interpretation of the results. Beyond the lack of regional development of these technologies, this study demonstrates that the use of these diagnostic methods is possible.

In a prospective study, Tarabichi et al. reported that the NGS technique reliably detected microorganisms in synovial fluid with a high degree of agreement with traditional cultures (96.1%);[20] in turn, it was found that NGS is a useful complement for the detection of pathogens in 81.8% of PPIs with negative culture.[13] In our series, there was agreement between cultures and NGS in eight of the 17 patients (patients 1, 3,4, 7, 8, 10, 13, 14); on the other hand, in another three, it was possible to identify a germ different from the one found in the cultures (cases 5, 12, 17), which modified the initial therapeutic behavior. Yin et al. described that the NGS technique has a sensitivity of 0.93 for the diagnosis of infection associated with a prosthesis, a value higher than that reported for the biomarkers CRP (0.67), interleukin 6 (0.47), procalcitonin (0. 67) and cultures (0.47), and these results were statistically significant ($p<0.05$). However, when evaluating specificity, NGS presented a value of 0.9, only higher than PCR (0.85; $p<0.05$).[13,23]

Although the described results of the application of NGS in the diagnosis of PJI are encouraging, several authors agree that it is necessary to validate these diagnostic methods with studies with a higher level of evidence and, in turn, evaluate the benefit-cost ratio.[24,25]

This study has limitations. Although its purpose was not to analyze the clinical outcomes, but rather the viability of using this novel technique, we believe that the patient sample is heterogeneous, which does not allow other conclusions to be drawn. In addition, no cost analysis was performed. On the other hand, its strength is its prospective design with meticulous data collection, in which samples of not only synovial fluid, but also of soft tissues were analyzed. It is important to note that the samples were analyzed in the center that has the largest genomic database in the world, a benefit when it comes to identifying the most atypical microorganisms and avoiding underdiagnosis.

CONCLUSIONS

According to our experience, the use of NGS in our field is viable as a tool for diagnosing PJI and the results are available in less than 72 hours, despite the distance from the analysis laboratory. Our findings suggest that some infections could be caused by other germs that escape conventional bacteriological detection. However, we believe that more studies are required to determine the role of NGS in the diagnostic algorithm and to understand the implication of certain rare microorganisms isolated in samples from patients who are apparently not infected.

Conflict of interest: The authors declare no conflicts of interest.

A. García-Mansilla ORCID ID: https://orcid.org/0000-0001-9820-8886
A. Albani Forneris ORCID ID: https://orcid.org/0000-0002-9463-2724
F. Díaz Dilernia ORCID ID: https://orcid.org/0000-0002-7830-2207
P. Slullitel ORCID ID: http://orcid.org/0000-0002-8957-075X
G. Zanotti ORCID ID: https://orcid.org/0000-0001-8090-4832
F. Comba ORCID ID: https://orcid.org/0000-0002-2848-2983
F. Piccaluga ORCID ID: https://orcid.org/0000-0002-9887-4886
M. Buttaro ORCID ID: https://orcid.org/0000-0003-3329-778X

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