

# Bulletin de la Dialyse à Domicile

## EVALUATION OF A NEW RAPID-DIAGNOSTIC TEST FOR PERITONITIS IN PERITONEAL DIALYSIS : The PERIPLEX® device

## EVALUATION D'UN NOUVEAU TEST DE DIAGNOSTIC-MINUTE DES PERITONITES EN DIALYSE PERITONEALE : Le dispositif PERIPLEX®

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### Résumé

En 2017, la société MOLOGIC® a mis au point un nouveau test de diagnostic rapide (PERIPLEX®) pour la détection des infections péritonéales chez les patients traités par dialyse péritonéale. Ce test à usage unique est basé sur la détection dans le dialysat de deux biomarqueurs inflammatoires : l'Interleukine-6 (IL-6) et la matrice metalloprotéinase-8 (MMP-8). Nous avons évalué ce test dans une étude multicentrique prospective regroupant 10 centres issus du RDPLF (Registre de Dialyse Péritonéale de Langue Française). Au total 184 tests ont été réalisés ; 86 tests étaient négatifs et 98 étaient positifs. 86 péritonites ont été confirmées. Il n'y a pas eu de faux-négatifs, et 12 faux-positifs ont été constatés. Parmi les 12 faux-positifs, 7 d'entre eux concernaient une situation de sepsis sans péritonite, ou d'inflammation péritonéale. Les performances du test sont jugées excellentes : sensibilité 100 %, spécificité 88 %, Valeur prédictive positive 88 %, Valeur prédictive négative 100 %. Dans cette étude, un test négatif permet d'éliminer formellement le diagnostic de péritonite.

Mots clés : Dialyse péritonéale, biomarqueurs, péritonite, infection, Interleukine-6, Metalloprotéinase-8, diagnostic

### Summary

In 2017, the British company MOLOGIC developed a new rapid-diagnostic test (PERIPLEX®) for the diagnosis of peritonitis in patients undergoing peritoneal dialysis. This single-use test is based on the detection in dialysate of two biomarkers of inflammation: Interleukin-6 (IL-6) and matrix metalloproteinase-8 (MMP-8). The test was evaluated in a prospective multicenter study including 10 centers from the RDPLF (French Language Peritoneal Dialysis Registry). A total of 184 tests were performed; 86 tests were negative and 98 were positive. 86 peritonitis were confirmed. There were no false-negatives, and 12 false-positives. Of the 12 false-positives, 7 of them were for sepsis without peritonitis, or peritoneal inflammation. The performance of the test is considered excellent: sensitivity 100 %, specificity 88 %, positive predictive value 88 %, negative predictive value 100 %. In this study, a negative test can formally rule out the diagnosis of peritonitis.

Key words : Peritoneal dialysis, biomarkers, peritonitis, infection, Interleukin-6, Metalloproteinase-8, diagnosis

## INTRODUCTION

Peritonitis is a common complication in peritoneal dialysis. Peritonitis is the worldwide leading cause of technique failure and transfer to hemodialysis [1]. In France peritonitis remains a dreaded complication with poor outcomes. Data from the French Language Peritoneal Dialysis Register (RDPLF) shows an overall rate of 1 episode / 36 patient-months, and a peritonitis-free survival of 50 % at 3 years. In 2018, peritonitis represented 14 % of the causes of technique failure in France [2, 3].

In peritoneal dialysis, the diagnosis of peritonitis is defined by the presence of at least two of the following three criteria [4]:

- Symptoms of peritonitis, for example abdominal pain and / or cloudy effluent dialysate.
- Cellularity of effluent dialysate >100 leukocytes / mm<sup>3</sup>, with >50% being polymorphonuclear cells.
- Positive dialysate effluent microbiological culture.

If peritonitis is suspected, the patient is usually called to the dialysis center to confirm the diagnosis, and to send a sample of the dialysate to the laboratory.

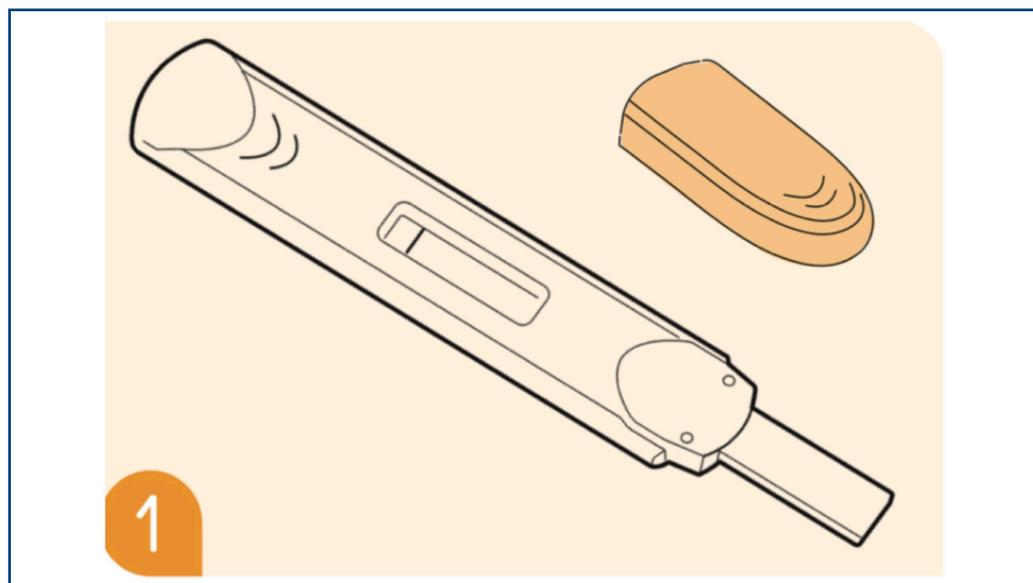
In order to limit the patient's movements, some centers first use urinary reagent strips at home as a rapid diagnostic test for peritonitis. These strips are based on the detection of esterase activity in granular leukocytes to assess the leukocyte concentration, and on the production of nitrites obtained by the activity of nitrates reductases from certain bacteria, mainly enterobacteria. Colorimetric reading by eye is imprecise, and may give rise to varying interpretations. Used for the diagnosis of peritonitis in peritoneal dialysis, urine reagent strips have been shown to give inconsistent results, with a sensitivity varying from 80 to 100 % depending on the studies, and a specificity varying from 45 to 95 % [5-8].

A new rapid point-of-care (POC) diagnostic test for peritonitis was developed in 2017 by the company Mologic Inc. working with Cardiff University and is distributed in France by MEDISUR. This test, called PERiPLEX®, is based on the detection, in dialysis effluent of Interleukin-6 (IL-6) and matrix metalloprotease-8 (MMP-8). Detection is performed by a lateral flow multiplexed immunoassay system. The cytokines IL-6 and MMP-8 are essential markers of inflammation and PMN activation, elevated in response to bacterial infections [9]. The objective of this study is to assess the intrinsic and extrinsic performances of this new device in a real world clinical setting.

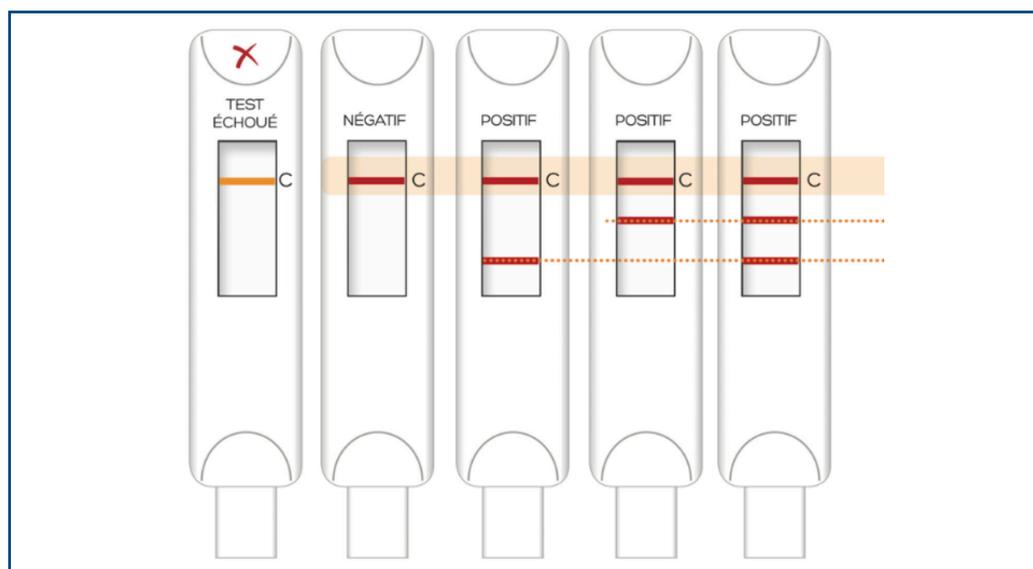
## MATERIAL AND METHODS

### Material

The PERiPLEX® device is presented as a classic single-use immunological diagnostic test, similar to the pregnancy tests. It has a wick at its end, to be dipped in the dialysis effluent to be tested (Fig. 1). The result is obtained within 5 minutes. The appearance of test bands indicates the presence of IL-6 and/or MMP-8 in the dialysate (Fig. 2). The test is positive if either IL-6 and/or MMP-8 lines appear. The procedure for performing the test and the instructions for use sent to each center is described at <https://www.prevention-addiction.pro/docs/PERiPLEX-notice.pdf>



↑Figure 1. Overview of the PERIPLEX® rapid diagnostic test device for peritoneal dialysis



↑Figure 2. Results and interpretation. Top row = Control; middle line = presence of IL-6; lower line = presence of

A total of 200 PERiPLEX® kits were provided free of charge by the French distributor, i.e. 20 kits per Center. In each center 5 tests were carried out in subjects free from peritoneal infection (“Control”) and 15 tests in subjects presenting with a suspicion of peritoneal infection.

### Methods

**Ethics:** This is a prospective multicenter clinical study evaluating a diagnostic medical device, bearing the European CE mark and not falling within the scope of prior authorization from the French Drug Administration (ANSM). The study falls outside the French Jardé law, with irreversible anonymization, without computerized data processing. The study was registered in the French General Data Protection Regulation (RGPD) on 04/03/2019 under reference P-006 PERIPLEX.

**Promoter:** ECHO. Expansion of Western Hemodialysis Centers. Pavillon Montfort, 85 rue St Jacques - 44000 NANTES

**Study duration:** one year, June 30, 2019 to June 30, 2020

**Selection of centers:** Ten centers were included among the 189 centers participating in the French Language Peritoneal Dialysis Registry (RDPLF). The centers were selected on the basis of the number of patients treated during the previous year, in order to have a sufficient number of events during the study period. The selection criterion used, depending on the number of patients treated and the annual number of events observed, was 15 to 20 tests expected over the study period, including 5 per center on uninfected subjects («Control»).

**Study design:** Except for the 5 “Control” tests, the PERiPLEX® test was to be carried out in each center, each time the healthcare team had decided to carry out a dialysate cell-count and bacteriological analysis on a drained bag for suspected peritoneal infection. The test was carried out on the dialysate from the same drained bag as that sent for analysis to the laboratory.

Dialysate cell-count and bacteriological analysis were performed according to ISPD recommendations [4], i.e. after a dwell of at least 2-hour duration. The maximum contact time before test was not specified and left to the choice of each center. When it was a test performed on the first drained effluent or after an empty peritoneal cavity period of more than 12 hours, the center identified this fact.

The analysis of the dialysate included total cell-count, direct examination, and microbiological culture in blood culture bottles.

The dialysate tested was treated the same if it was from APD or CAPD patients.

**Data processing:** No data identifying patients was carried collected. The anonymization was complete and irreversible. The data collected were:

- The results of the dialysis effluent cell-count.
- The results of the bacteriology of the dialysis effluent.
- The results of the reagent urinary strip (if carried out)
- The results of the PERiPLEX® : positive or negative according to manufacturer’ manual
- An evaluation of the ergonomics of the device On a 5-item scale assessment

**Data transmission:** The data were recorded by the investigators manually on a paper CRF (Case Report Form) (Fig. 3) at the rate of one sheet per PERiPLEX® test used. Each CRF was immediately sent to the RDPLF coordinating physician and recorded in an Excel spreadsheet. In the event of an anomaly, the investigator was called back by the co-ordinating physician.

**Evaluation des performances du test et analyses statistiques :** Les performances intrinsèque et extrinsèque du test ont été analysées à partir d’un tableau de contingence. La Valeur Prédictive Positive a été calculé par la formule de Bayes, à partir de la sensibilité, de la spécificité et de la prévalence des péritonites dans l’ensemble des tests réalisés. L’analyse statistique a utilisé le coefficient Q de Yules, le CHI-2 et l’indice de Youden.

Suspicion de péritonite :		OUI <input type="checkbox"/>	NON <input type="checkbox"/>
Dialysat trouble :		OUI <input type="checkbox"/>	NON <input type="checkbox"/>
Bandelette réactive :		Leucocytes :	
		Nitrites :	
Test PERIPLEX	POSITIF <input type="checkbox"/>	NEGATIF <input type="checkbox"/>	
<b>EXAMEN CYTO-BACTERIOLOGIQUE DU DIALYSAT :</b>			
Cellularité globale (par mm3):			
Formule :			
- Neutrophiles (en pourcentage) :			
- Eosinophiles (en pourcentage) :			
- Autres (en pourcentage) :			
Bactériologie :			
- Examen direct :			
- Culture :			
<b>COMMENTAIRES (FACULTATIFS)</b>			
Au final, y a-t-il eu infection :		péritonéale <input type="checkbox"/>	autre <input type="checkbox"/>
		NON <input type="checkbox"/>	
L'utilisation du dispositif PERIPLEX vous a-t-elle paru :			
Très facile	Facile	Moyenne	Difficile
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			Très difficile
			<input type="checkbox"/>

↑ Figure 3: Case Report form (CRF) completed during the test and sent to the RDPLF coordinating physician.

## RESULTS

### Selected centers

The 10 RDPLF participating centers meeting the selection criteria were:

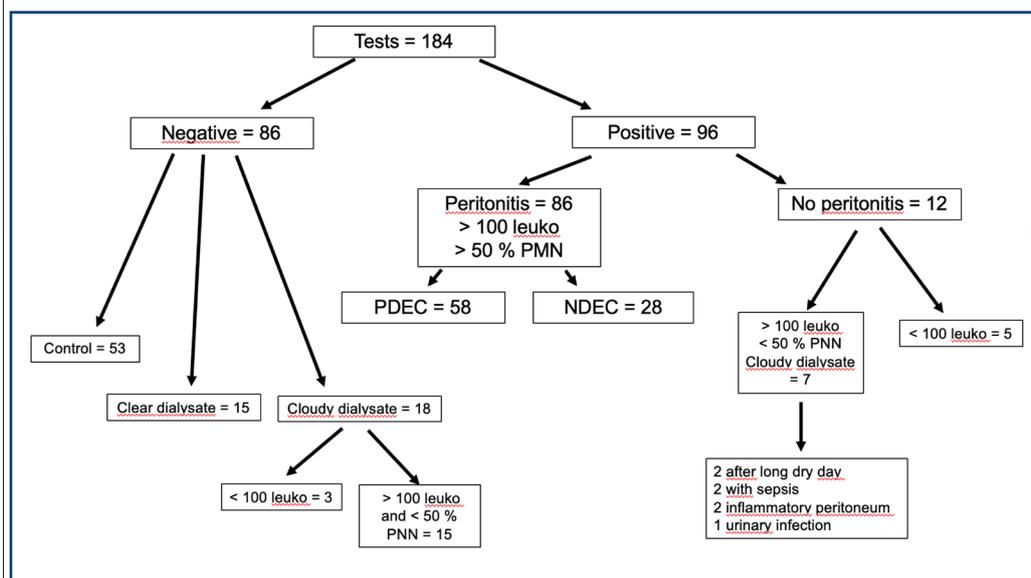
Brussels (Belgium), Caen (France), Corbeil-Essonnes (France), Dunkerque (France), La Roche sur Yon (France), Lille (France), Louvain (Belgium), Nice (France), Noumea (New Caledonia), Vichy (France).

Doctors from the selected centers all agreed to participate in the study. In accordance with the RGPD (French General Data Protection Regulation), an information letter has been sent to the administration of their establishment.

### Overall results

A total of 184 PERiPLEX® tests were performed on 184 different dialysate samples. Of the 184 tests performed, 53 were «Control i.e. performed on dialysate from patients without any clinical

evidence of peritoneal infection. 131 tests related to suspected peritonitis; 86 of these peritonitis episodes were confirmed according to the official definition [4]. The PERIPLEX® tests were all negative for the “Control” samples, and all were positive for confirmed peritonitis. Among the 86 peritonitis, the bacteriological culture of the dialysate was positive for 58 of them (PDEC), and culture negative for the other 28 (NDEC) (Fig. 4).



↑ Figure 4. Global results of PERIPLEX® tests. (Leuko = Leukocytes / mm<sup>3</sup>; PMN = Polymorphonuclear cells; PDEC = Positive dialysis effluent culture; NDEC = Negative dialysis effluent culture)

The PERiPLEX® test were positive 12 times in the absence of clinically defined peritonitis (false positives). Of these 12 samples, 7 of the dialysates were cloudy, and had >100 leukocytes/mm<sup>3</sup>. Of these 7 samples of cloudy dialysate, 2 were collected after a period when the peritoneal cavity had been without fluid of more than 12 hours in 3 cases there was evidence of ongoing infection (fever, tunnel infection or urinary tract infection); in 2 cases there was evidence of peritoneal inflammation of other etiology (e.g. strangulated hernia, mesenteric ischemia). There were no false-negative tests (Table 1).

↓ Table 1. Results of the tests. Contingency table.

	Péritonitis	No peritonitis	Total tests
Positive tests	86	12	98
Negative tests	0	86	86
<b>TOTAL</b>	86	98	184

#### Evaluation of intrinsic and extrinsic performance of the test

The performance of the test is shown in Table 2.

↓Table 2. Test performances

<b>Sensitivity</b>	100 %
<b>Specificity</b>	87.8 %
<b>Positive predictive value</b>	87.8 %
<b>Negative predictive value</b>	100 %
<b>False positive rate</b>	12.2 %
<b>False negative rate</b>	0 %
<b>Peritonitis rate in the whole set of performed tests</b>	46.2 %
<b>Rate of positive test in the whole set of performed tests</b>	53.3 %
<b>Youden index</b>	0.88
<b>Yule Q coefficient</b>	1
<b>X<sup>2</sup> (CHI-2)</b>	141.7

### Ergonomics of the test

Tests users were asked to comment on the ease of use of the device (Fig. 4). The opinion was rated according to 5 ratings: Very easy; Easy; Neither easy or difficult; Difficult ; Very difficult. Of the 184 tests carried out, we received 158 responses, of these 97.5 % of them were rated “Very easy” or Easy”; 3 were rated “neither easy or difficult” and 1 “Difficult” (Table 3).

↓Table 3. Responses to the questionnaire to assess the ease of use of the test (N = 158)

<b>Ease of use</b>	<b>N = 158</b>
Very easy	83
Easy	71
neither easy nor difficult	3
Difficult	1
Very difficult	0

## **DISCUSSION**

This study is the largest study (N = 184) conducted to date of a rapid point-of-care diagnostic tests for peritoneal infections in peritoneal dialysis patients. Previous studies were carried out using urine test strips, with heterogeneous performance in the literature: sensitivity varying from 80 to 100 % depending on the study, specificity varying from 45 to 95 % [5-8]. The results of this study on the PERiPLEX® test show excellent performance, with a sensitivity of 100 %, a specificity of 88 %, a Positive Predictive Value of 88 %, a Negative Predictive Value of 100 %. The absence of false-negatives in the 184 tests carried out, makes it possible to formally rule out peritonitis if the test is negative

The advantage of a simple rapid diagnostic test for the diagnosis of peritonitis in peritoneal dialysis, is its use at home by the patient himself in case of the suspicion of infection. A negative test should help reassure the patient and avoid unnecessary travel to the hospital. In this study, the test was performed by nurses, but its simplicity and ease of use should allow use by the patients or carers themselves (Tab. 3). Further studies should confirm this point.

Reactive urine strips, optional in the study protocol, were not included in the analysis. The uri-

nary reagent strips are based on the detection of esterase activity in granular leukocytes to assess the leukocyte concentration, and on the production of nitrites obtained by the activity of nitrates reductases of certain germs, mainly enterobacteria. The colorimetric reading by eye of these reagent strips can lead to different interpretations, explaining the great heterogeneity of the results in the literature [5, 8].

Before marketing, the manufacturer tested the PERiPLEX® device on 121 frozen samples of drained dialysate, from patients undergoing peritoneal dialysis, 66 with peritonitis and 55 without peritonitis. Its sensitivity was evaluated at 98.5 % and its specificity at 94.5 % (Manufacturer data, unpublished). In 2020, Goodlad evaluated the PERiPLEX® test in an outpatient setting on 107 dialysate samples, including 41 peritonitis [10]. In this study, the sensitivity and specificity were 97.6 % and 87.7 % respectively, with a Positive and Negative Predictive Value of 83.7 % and 98.3 % respectively [10].

It should be noted, in our study, a significant proportion of aseptic (culture negative) peritonitis (32%) that is higher than the overall national average of 17% noted in the RDPLF [11]. During the study period, the centers included recorded in the RDPLF a total of 143 peritonitis (i.e. 1 episode / 31 patient-months), and a rate of aseptic peritonitis of ~20 %. In our study, 86 peritonitis episodes were identified. As a result, not all peritonitis episodes were tested with PERiPLEX®. Due to chance, the test was certainly performed more frequently for aseptic peritonitis, in centers which already had a high rate of aseptic peritonitis, as a 20 % rate of aseptic peritonitis is higher than the national average, and above the targets recommended by the ISPD [4]. This confirms the need for each center to have techniques sensitive enough to detect up to 1 CFU / mL, which modern microbiology must now allow [12].

Unlike urinary reagent strips, the markers used by PERiPLEX® are specific to inflammation [9].

The PERiPLEX® test is based on the detection, in the effluent dialysate, of Interleukin-6 (IL-6) and of matrix metalloprotease-8 (MMP-8). According to the manufacturer's recommendations, the test is considered positive if IL-6 and / or MMP-8 is detected, without distinction between these two markers (Fig. 2). In our study, the test was interpreted according to the recommended procedure. However, it would have been interesting to distinguish, among the false positives, IL-6 positives alone, MMP-8 positives alone, and IL-6 + MMP-8 positives. These data were not available in this study.

MMP-8 is an inflammatory mediator found in high concentrations in sepsis with a digestive tract origin [13]. Using self-learning algorithms among 49 biomarkers tested during peritonitis in peritoneal dialysis, MMP-8 was found in the top 5 biomarkers to distinguish bacterial peritonitis, sterile peritoneal inflammation, or viral infection [14].

In the event of systemic inflammation, plasma IL-6 increases, and is found in the peritoneal dialysate after crossing the peritoneal membrane [10, 15, 16]. Therefore, it is possible that in the absence of peritonitis, systemic inflammation may impact positively on the test. Of the 12 false-positives found in this study, 7 of them concerned situations of sepsis without peritonitis, or peritoneal inflammation. Similar results were reported in the Goodlad study [10].

## CONCLUSION

This new test, based on the direct detection of biomarkers of peritoneal infection, is a simple, reliable, rapid test that can be performed easily by patients themselves at home in the event of a suspected peritonitis. The statistical indices for evaluating the diagnosis value of the test are excellent. The Yule Q coefficient, which measures the strength of the link between the test and peritonitis, is at the maximum value of 1. The Youden index of 0.88 confirms the remarkable effectiveness of this test. Based on the results of this study, a negative test on cloudy dialysate ruled out the diagnosis of peritonitis. The PERiPLEX® test represents, in 2020, a reliable rapid test for peritonitis diagnosis in patients undergoing peritoneal dialysis.

## DISCLOSURES

- the authors have no conflict of interest with the company MOLOGIC or the company MEDISUR.
- PERiPLEX® tests were provided free of charge by MOLOGIC and its French distributor MEDISUR.
- no funding was granted for this study, none of the participants and co-author were remunerated.
- the RDPLF benefited in 2019 from a co-sponsorship from Mologic for the organization of the 15th RDPLF Symposium ([https://www.rdplf.org/images/PDF/PROGRAMME\\_SYMPO\\_def.pdf](https://www.rdplf.org/images/PDF/PROGRAMME_SYMPO_def.pdf))

*Roles of authors and participants:*

*Pierre Yves Durand designed the protocol and carried out the administrative procedures for compliance with good ethical practices, carried out the statistical analysis and its interpretation.*

*Christian Verger was the initiator of the study and ensured the collection and quality control of information, participated in writing the article.*

*The physician and nurses of the centers ensured the smooth running of the protocol in their respective centers.*

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