



Histopathology of peritoneal adhesions in a Congolese female population *Histopathologie des adhérences péritonéales dans une population féminine congolaise*

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Résumé

Contexte et objectif. La composition des adhérences péritonéales est une préoccupation en pratique courante. L'histopathologie permet toutefois d'élucider ce mystère en orientant le diagnostic étiologique, notamment l'endométriose et d'autres déterminants. L'étude a décrit le profil histopathologique des adhérences péritonéales et identifier les facteurs associés dans notre milieu. **Méthodes.** Etude transversale, menée aux CUK du 1^{er} mars au 30 juin 2013, incluant les femmes ayant subi une laparotomie ou une laparoscopie et qui présentaient des adhérences péritonéales. La comparaison entre les moyennes a été faite à l'aide du test *t* de Student, et celle entre les proportions par les tests Chi-carré de Pearson et Exact de Fisher. Pour les données non-paramétriques, le test U de Mann-Whitney a été utilisé. **Résultats.** Au total 45 patientes (34,5 ± 6,9 ans) étaient examinées. L'âge moyen des adhérences était de 2,7 ans avec des extrêmes allant de 0,1 à 26,9 ans. L'inflammation était retrouvée dans 84,4 % d'adhérences. La réaction à corps étranger était présente dans 8,9 % d'adhérences. Les patientes sans antécédent de laparotomie avaient statistiquement plus d'inflammation dans leurs adhérences que les autres patientes (p=0,003), et celles qui présentaient une cicatrice hypertrophique avaient moins de cellules dans leurs adhérences que les autres (p=0,023). **Conclusion.** L'histologie des adhérences est dominée par les éléments inflammatoires ; plus prédominants chez les opérées sans antécédent de laparotomie, suggérant une origine infectieuse de ces adhérences. Cette composition varie aussi en fonction de l'existence ou non d'une cicatrice hypertrophique.

Mots-clés : adhérences péritonéales, histologie, composition

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Summary

Context and objective. The composition of peritoneal adhesions remains a concern for the practitioner. The environmental components, in conjunction with the epidemiology of certain conditions such as endometriosis, pelvic and the use of some treatments such as peritoneal dialysis and radiotherapy, may alter this composition. The study of adhesion histopathology in our environment could make it possible to determine the place of certain pathologies such as endometriosis, but also the involvement of some factors in their composition. The objective was to describe the histopathology of peritoneal adhesions and to identify associated factors in our practice. **Methods.** A cross-sectional study was conducted at the Department of Obstetrics and Gynecology of the Kinshasa University Hospital from 1st March to 30th June 2013 including women who underwent laparotomy or laparoscopy and who presented peritoneal adhesions. The statistics were performed using the SPSS 21 software. The comparison between means was analyzed using Student *t*-test and between proportions using the Pearson Chi-square and Fisher Exact tests. For non-parametric data, the Mann-Whitney U-test was used. **Results.** The mean age ± standard deviation of 45 patients was 34.5 ± 6.9 years. Inflammation was found in 84.4% of adhesions. The foreign body reaction was present in 8.9%. Patients without a prior laparotomy had statistically more inflammation in their adhesions (p = 0.003), and those with hypertrophic scars had fewer cells in their adhesions (0.023). **Conclusion.** The histology of adhesions is marked by inflammatory elements; more predominant in patients without prior laparotomy, suggesting an infectious origin of these adhesions. This composition also varies according to the presence or not of a hypertrophic scar.

Keywords: peritoneal adhesions, adhesions histology, adhesions composition

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Introduction

The composition of peritoneal adhesions remains a concern for the practitioner. The usefulness of its microscopic description became apparent almost two decades ago (1-2) and helped to improve the knowledge about their pathophysiology. This composition, as well as the prevalence and severity of peritoneal adhesions, are strongly influenced by the environment.





These include the human environment, the practice of microsurgery principles by practitioners, and the operating room physical environment (use of gloves with talc, presence of air particles, presence of foreign bodies in the surgical field). These environmental components, in conjunction with the epidemiology of certain conditions such as endometriosis, pelvic infections especially with *Chlamydia trachomatis* (3-5), and the use of some treatments such as peritoneal dialysis and radiotherapy, may alter this composition.

The study of adhesion histopathology in our environment could make it possible to determine the place of certain pathologies such as endometriosis, but also the involvement of some factors in their composition. In this respect, we previously carried out a study (6) on the epidemiology of peritoneal adhesions which showed a possible relationship between uterine fibroid, skin scar abnormalities and adhesions. The same study reported 13.46 % of adhesions in patients without a history of abdominal surgery, suggesting an inflammatory origin.

The objective of this study was to describe the histopathology of peritoneal adhesions and to identify associated morphologic factors in our setting.

Methods

A cross-sectional study was conducted at the Department of Obstetrics and Gynecology of the University Hospital of Kinshasa (UHK). It included patients who had peritoneal adhesions during a laparotomy or laparoscopy performed from March 1st to June 30th, 2013. Patients treated for peritonitis and for cancer were excluded.

Socio-demographic data, medico-surgical and gynecological history, perioperative and pathological variables were recorded.

For each patient enrolled in the study, the data were collected by interview. The intraoperative findings were recorded during the procedure and histopathological characteristics were recorded

by microscopic analysis using a pre-established data sheet.

The economic level, previous abdominal surgery, age of adhesions, skin scar anomaly and ASG classification were defined as previously reported (6).

Inflammation has been defined by the presence of inflammatory cells (mononuclear, polynuclear) and edema.

The foreign body reaction consisted either as a typical granuloma with a foreign body or only as giant multinucleate cells surrounding a foreign body but not organized in a typical granuloma.

Intraoperative description and sampling of adhesions

During surgery, the surgeon noticed the presence or absence of adhesions. In case of adhesions, their number, the organs involved and their type for the classification according to the ASG were noticed.

Following the description, the surgeon sampled 5 to 10 mm of adhesions at one site if there was only one adhesion or at the most severe site if there were several adhesions, specifying the origin of each tissue and the organs involved.

Histopathological examination

The sampled adhesions were immediately fixed in 10% phosphate-buffered formaldehyde and transferred to the pathology laboratory of the UHK. Once in the laboratory, samples were taken from the surgical specimen, which was placed in suitable cassettes for tissue treatment. This treatment included dehydration in four successive alcohol baths at 60, 80, 90, and 100%, respectively, for one hour in each bath. This process was followed by fragments immersion in three successive xylol baths for 15, 30 and 60 minutes. After this step, they were put in a paraffin bath in the incubator at 57° for 12 hours. The embedding of the fragments was done in paraffin wax to form blocks which were cut with a microtome. Sections of 5 µm were stained with Hematoxylin-Eosin and Masson Trichrome. This staining was done in the same



time period for all the samples in order to reduce the internal variations of colorations. When staining with Hematoxylin and Eosin, the number of cells (mononuclear cells, fibroblasts, adipocytes, endothelial cells) was evaluated using the method reported by Binnebösel *et al.* (1): 0 = 0% cells, 1 = 1 to 20%, 2 = 21-50%, 3 = 51-80%, 4 = 81-100%. The percentage was considered in relation to 40 x magnification microscopic fields.

The histopathological characteristics were studied separately by two examiners who were blinded for patients' characteristics.

Statistical analysis

The data were analyzed using Statistical Package for Social Sciences (SPSS) 21 software. The Pearson Chi-square and Fisher's Exact tests were used to compare proportions. For parametric data, with normal distribution, means were compared by Student t test. The difference between groups for the non-parametric data was analyzed by the Mann-Whitney U-test. The value of p less than 0.05 was considered statistically significant.

Ethical Considerations

The study protocol was approved by the ethics committee of the School of Public Health of the University of Kinshasa (reference ESP / CE / 064/13). Informed and written consent was obtained from the patients prior to their inclusion in the study.

Results

Patients' Characteristics

Overall, 45 patients were included in the study. The mean age \pm SD was 34.5 ± 6.9 years. Of these patients, 53.3% had a university level and 51.1% were moderately rich.

Among the patients, 28.9 % and 8.9% respectively had hypertrophic and keloid scar, 15.6% had no history of abdominal surgery, and 42.5% were diagnosed with uterine leiomyoma or had leiomyoma diagnosed during surgery (Table 1).

Table 1. Patient's characteristics

Variables	
Age (years) of patients, (means \pm SD) (n=44)	34.5 \pm 6.9
Educational level, n (%)	
Primary	1 (2.2)
Secondary	20 (44.4)
University	24 (53.3)
Economic level, n (%)	
High	15 (33.3)
Mild high	23 (51.1)
Average	6 (13.3)
Low	1 (2.2)
Skin scar anomaly, n (%)	
Hypertrophic scar	
Yes	13 (28.9)
No	32 (71.1)
Keloid	
Yes	4 (8.9)
No	41 (91.1)
Previous abdominal surgery, n (%)	
Yes	38 (84.4)
No	7 (15.6)
History/presence of leiomyoma, n (%), (n=40)	
Yes	17 (42.5)
No	23 (57.5)

SD: Standard Deviation

Adhesions characteristics

General characteristics

The mean age of adhesions was 2.7 years, ranging from 0.1 to 26.9. Nearly two-thirds of adhesions (63.2%) were at least 2 years old and 88.9% were middle according to the ASG classification (Table 2).

Pathological features

Hematoxylin-Eosin and Masson Trichrome staining characteristics of adhesions

By Hematoxylin-Eosin staining, 53.3% of adhesions were made of loose connective tissue. Inflammation was found in 84.4% of cases with 60% of the slides containing the mononuclear cells and 36.6% of the polymorphonuclear cells. Overall, 60% of the slides contained adipocytes and 31.1% had a proportion of adipocytes between 21 and 50%. All adhesions had vessels



and more than a half had a moderate amount of these structures (Figure 1).

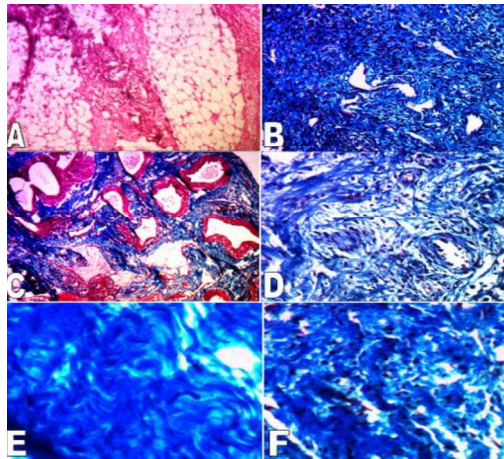


Figure 1. Adhesion composition. A: HE staining showing a connective tissue with a large amount of adipose tissue. B: trichrome staining. Dense adhesion connective tissue with high cellularity. C: trichrome staining. Adhesion connective tissue containing a large amount of vessels with hypertrophic wall. D: trichrome staining showing dense connective tissue with fibroblast cells. E: trichrome staining. Collagenic fibrosis. F: trichrome staining. Combined Fibroblastic and collagenic fibrosis. (original magnification, 100x in A – C and 400x in D – F)

The foreign body reaction (figure 2) was present in 8.9% of adhesions.

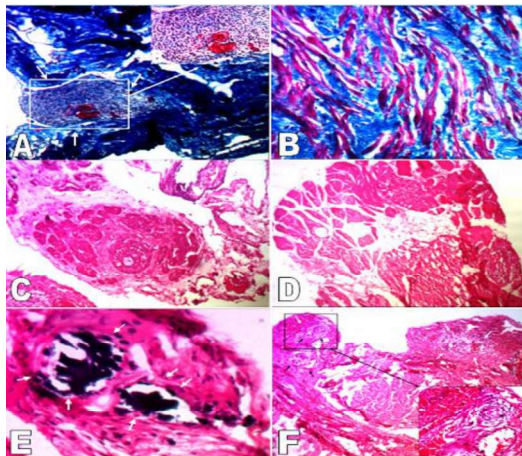


Figure 2. HE and Masson's trichrome staining features of unusual findings on adhesions. A: cytotrogen stroma in 100 x Masson's trichrome (arrows, zoom on the right upper corner). B: 400 x Masson's trichrome of smooth muscle development within connective tissue of adhesions. C: Neuroid hyperplasia associated with connective tissue of adhesions (100 x HE). D: 100 x HE staining of skeletal muscle continuing with connective tissue of adhesions. E: 400 x HE staining of calcifications. F: 100 x HE staining of skeletal muscle continuing with connective tissue of adhesions.

(arrows). F: Foreign body granuloma with HE, 100 x magnification. (arrows, zoom on the right downer corner)



By Masson Trichrome staining, adhesions had a significant amount of fibers (at least 2+) in 66.6% of cases. According to the presence of fibroblasts, 64.4% had an amount varying between 1 and 20% per field and fibrosis was significant (at least 2+) in 17.8% of adhesions.

In addition to the foreign body reaction, other non-usual findings (figure 2) within the adhesions were noticed in 6 cases (13.3%). These included 2 cases of non-vascular smooth muscle, 2 cases (4.4%) of Skeletal muscle, with calcification and cytogenic chorion, 1 case (2.2%) of neuroidal nerve hyperplasia and 1 case of ovarian stroma.

In bivariate analysis associating different groups of patients and the pathological characteristics of the adhesions (evaluated by number of +). Table 3 shows that the history of laparotomy was associated with severe inflammation. Similarly, hypertrophic scar was associated with the large number of cells in the adhesions. Indeed, patients without a history of laparotomy had statistically more inflammation in their adhesions compared to other patients ($p = 0.003$), and those with a hypertrophic scar had fewer cells in their adhesions than the others, in a statistically significant way ($p=0.023$).



Table 2. Adhesions characteristics

General characteristics					
Age of adhesions (n=38)		n	%	Median (range)	
< 2 years		14	36.8	2.7 (0.1 – 26.9)	
≥ 2 years		24	63.2		
Classification ASG					
Mild		40	88.9	N/A	
Moderate		5	11.1		
Hematoxylin – Eosin staining					
Cells			Other characteristics		
Mononuclear cells		n	%	Type of adhesion tissue	
Absent		18	40.0	Loose connective tissue	
1 - 20 %		21	46.7	Dense connective tissue	
21 - 50 %		6	13.3	Magnitude of Inflammation	
Polynuclear cells				No inflammation	
Absent		29	64.4	+	
1-20 %		11	24.4	++	
21 - 50 %		1	2.2	+++	
51 - 80 %		3	6.7	Blood vessels	
81 - 100 %		1	2.2	+	
Adipocytes				++	
Absent		18	40.0	+++	
1 - 20 %		9	20.0	Foreign body reaction	
21 - 50 %		14	31.1	No FBR	
51 - 80 %		3	6.7	Foreign body giant cells	
81 - 100 %		1	2.2	Foreign body granuloma	
Masson trichrome staining					
Fibers quantity		N	%		
+		15	33.3		
++		28	62.2		
+++		2	4.4		
Fibroblasts quantity					
1-20 %		29	64.4		
21 - 50 %		16	35.6		
Magnitude of fibrosis					
No fibrosis		13	28.9		
+		24	53.3		
++		7	15.6		
+++		1	2.2		

FBR: Foreign Body Reaction, N/A: Not applicable



Table 3. Association between patients' groups and HE/Masson's trichrome staining characteristics of adhesions

Groups	Hematoxylin et Eosin staining			Masson' s Trichrome staining			
	Quantity of cells Median (IOR)	Magnitude of inflammation Median (IOR)	P value	Quantity of vessels Median (IOR)	Quantity of fibers Median (IOR)	Magnitude of fibrosis Median (IOR)	P value
Age of adhesions (years)*							
< 2	2+(1+ - 2+)	1+(0+ - 2+)	0,697	2+(1+ - 3+)	2+(1+ - 2+)	1+(0+ - 2+)	0,401
≥ 2	2+(1+ - 3+)	1+(0+ - 2+)	0,449	1+(1+ - 3+)	2+(1+ - 3+)	1+(0+ - 3+)	0,647
History of laparotomy							
Yes	2+(1+ - 3+)	1+(0+ - 2+)	0,055	2+(1+ - 3+)	2+(1+ - 3+)	1+(0+ - 3+)	0,927
No	2+(2+ - 3+)	2+(1+ - 3+)	0,003	2+(1+ - 2+)	2+(1+ - 2+)	0+(0+ - 1+)	0,622
Keloid							
Yes	2+(2+ - 2+)	1+(1+ - 2+)	0,695	1,5+(1+ - 2+)	2+(1+ - 2+)	1,5+(1+ - 2+)	0,71
No	2+(1+ - 3+)	1+(0+ - 3+)	0,633	2+(1+ - 3+)	2+(1+ - 3+)	1+(0+ - 3+)	0,833
Hypertrophic scar							
Yes	2+(1+ - 2+)	1+(0+ - 2+)	0,023	1+(1+ - 3+)	2+(1+ - 3+)	1+(0+ - 3+)	0,923
No	2+(1+ - 3+)	1+(0+ - 3+)	0,511	2+(1+ - 3+)	2+(1+ - 3+)	1+(0+ - 2+)	0,691
History and/or presence of leiomyoma							
Yes	2+(1+ - 3+)	1+(0+ - 2+)	0,798	2+(1+ - 3+)	2+(1+ - 3+)	1+(0+ - 2+)	0,975
No	2+(1+ - 3+)	1+(0+ - 2+)	1	2+(1+ - 3+)	2+(1+ - 3+)	1+(0+ - 3+)	0,772



Discussion

Characteristics of Patients

In the present study, 28.9% of patients had hypertrophic scars compared to 8.9% of keloid scars. The proportion of hypertrophic scar is similar to that of 29.17% reported by Mushegera *et al.* (7) on the general population in the same setting. However, the proportion of keloids is lower than that of 13.5% by the same authors. Nevertheless, this proportion of keloid is slightly higher than 7.1% reported by Tulandi *et al.* (8) in a population of African American women undergoing a cesarean section.

Adhesions were found in 15.6% of women with no history of laparotomy. These adhesions, described by Weibel *et al.* (9) as "spontaneous", are secondary to infectious or inflammatory pathology such as endometriosis (2 cases in the present study), radiotherapy and peritoneal dialysis (4-5). This proportion of "spontaneous" adhesions is less than 28% reported by Weibel *et al.* (9), but greater than 10.4% noted by Menzies *et al.* (10). The high frequency in the Weibel study is probably related to the fact that it was an autopsy study making certainly easier for the researchers to search for adhesions in the cadavers. Compared to Menzies *et al.* (10), the higher proportion of the present study would probably be related to the higher prevalence of infectious diseases in our environments.

Adhesions characteristics

Age of adhesions

The adhesions median age was 2.7 years, higher than the one reported by Binnebösel *et al.* (1) (1.5 years) and by Luijendijk *et al.* (11) (1.1 years). The age of adhesions can influence their prevalence and composition. Indeed, it has been reported a possible spontaneous resorption of recent adhesions although in most cases these adhesions persist even decades after their occurrence (1). And over time, the composition of adhesions can be modified in particular by the resorption of some materials such as suture and

other foreign bodies, and by the replacement of fibrosis with adipose tissue (1).

Hematoxylin-Eosin and Masson Trichrome staining characteristics of adhesions

The composition of adhesions was remarkable by a predominance of adipocytes (60% of samples), significant inflammation (84.4% of cases), a large amount of blood vessels (100 % slides), foreign body reaction in 8.9% and unusual findings in 13.3 % of cases.

Adipocytes were statistically more present in older adhesions compared to the more recent ones. This finding is in agreement with that noted by Binnebösel *et al.* (1). A reduction in the remodeling activity has been described in the adhesions over time, which could explain the decrease in the amount of fibroblasts and thus of fibers, being gradually replaced by adipocytes. All adhesions in the present study contained blood vessels, consistent with the findings of Sulaiman *et al.* (12). These results contrast with those of 30% of blood vessel reported by Binnebösel *et al.* (1). The difference between the studies could be due to the types of adhesions described in each study and to the development in the description of adhesions. Indeed, some ancient authors described adhesions as avascular fibrous bands (1, 13-14). It was therefore necessary to count with the development of certain techniques of staining and the introduction of immunohistochemistry in order to see the knowledge on the composition of adhesions increase considerably.

Inflammation was predominant in 84.4% of cases. This important inflammation could be explained by the probable implication of infectious factors, and hence of the immunity, in the onset of these adhesions in our setting. Moreover, the existence of chronic inflammation in adhesions could contribute to the consumption of some proteins of inflammation, namely the complement factors whose involvement in adhesions has already been demonstrated.



Some unusual findings were noticed, including the presence of smooth muscle tissue. This finding could be related to the involvement in the adhesions, of the smooth muscular layer of some abdominal organs and to the recognized transformation of fibroblasts which express a smooth muscular configuration with the synthesis of actin-myosin smooth muscle.

The foreign body reaction was present in 8.9% of cases, with only one well-formed granuloma (2.2%). The study conducted by Luijendijk *et al.* (11) on foreign bodies in postoperative adhesions reported 26% of foreign body granulomas. The difference could be related to the age of adhesions that is different in both studies and to the fact that some samples in the present study could have been done on adhesions that did not include foreign bodies. In terms of age, there has been recognized that foreign bodies, as well as the reaction they cause, can be resorbed overtime. This reduces the chance of finding this reaction in older adhesions, granulomas being frequently seen in recently operated patients (11).

Factors associated with histopathological characteristics of adhesions

In the present study, patients without a history of laparotomy had more inflammation in their adhesions than others ($p = 0.003$). The inflammatory (the infectious) etiology of the adhesions found in these patients without prior laparotomy could explain this result. This association between inflammation and the absence of previous surgery once again raises the issue of the role of infection in the adhesion formation.

Among putative pathogens, *Chlamydia trachomatis* (CT) is the most incriminated in the genesis of peritoneal fibrosis (15) resulting in chronic pelvic pain, infertility and ectopic pregnancies (16). The damage responsible for these complications is secondary to the persistence of the pathogen in the genital tract (17). This persistence is promoted by an

immunological escape by the pathogen, losing a surface antigen; the Major Outer Membrane Protein (MOMP), against which the host produced antibodies. In this form, the pathogen synthesizes Heat Shock Proteins (HSP) in order to preserve its DNA integrity. Among these proteins is HSP-60 (18-19, 20), which is highly immunogenic and induces an important synthesis of antibodies by the host (17, 21- 22). The significant similarity between the amino acid sequence of the HSP - 60 of the CT and that of the host cells (22) would explain some autoimmune reactions and the magnitude of tubal fibrosis (17, 21). This led some authors to propose the use of CT anti-HSP serology as a less invasive and less expensive method for predicting tubal obstruction in infertility (21, 23). We (24) reported an association between hysterosalpingographic and laparoscopic tubal anomalies and the anti-HSP antibody titers of CT in infertile women.

Regarding the cell composition of adhesions, patients with hypertrophic scars had statistically fewer cells in their adhesions compared to other patients ($p = 0.023$). Otherwise, these patients had more extracellular matrix than those without hypertrophic scars. This observation reinforces our previous observation on the possible association between skin scar anomalies and the occurrence of adhesions (6). Indeed, there is a common micro-environment in many of these fibrotic conditions characterized by an excess of production and deposition of the extracellular matrix (25-28).

The low proportion of operated patients with keloids in this study did not allow conclusions to be drawn about its relationship to adhesion composition. However, subsequent studies in our setting could make it possible to exploit this pathway on the possible prediction of keloid scar on the occurrence of adhesions.

The results in the present study have to be analyzed considering the limitation of the adhesion sampling method. In fact, the tissue sample covering 10 mm of adhesions could not



be guided otherwise. This may have left in place important characteristics which required a description in this study. This fact could lead to a partial analysis of the reality with regard to the composition of adhesions.

Nevertheless, the present study is the first one in our setting, describing the histopathology of adhesions and made it possible to establish a relationship between some characteristics of patients and the composition of adhesions. As such, it is considered to be a pilot work to guide further studies in this area.

Conclusion

Postoperative adhesions are the most encountered adhesions in our practice. Their histopathology is dominated by inflammation; the latter predominated in patients without history of laparotomy, suggesting an infectious etiology. The composition differed between patients with and without hypertrophic scar, supporting the similar molecular environment between adhesions and some fibrotic conditions, namely skin scar anomaly.

Conflict of interest

The authors declare no conflict of interest

Authors' contribution

Nzau-Ngoma E: participated to the conception of the research, conducted data collection, performed statistics and wrote the final version of the work
Lebwaze BM: participated to the conception of the research, conceived the pathological method, read the final version

Mbuyi-Muamba JM: participated to the conception of the research, read the final version

Mboloko EJ: participated to the conception of the research, read the final version

Arung WK: participated to the conception of the work and read the final version

All authors approved the final and revised version of the manuscript

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