EVAR and OPEN treatment of abdominal aortic aneurysm: What is the role of MMP-9 in the follow-up?

Traitément de l’anévrysme de l’aorte abdominale par endoprothèse ou chirurgie ouverte : quel est le rôle de la MMP-9 au cours du suivi ?

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Summary
Introduction. — The aim of our study is to verify the role of metalloproteinases in endovascular repair (EVAR) and OPEN surgery treatment for abdominal aortic aneurysm (AAA). Postoperatively, these enzymes could represent an important biomarker to adapt diagnostic tests and further investigations during follow-up.

Material and method. — From 2004 to 2008, 55 patients were considered with AAA. Of these, 33 patients (mean age: 70.1 years), (mean AAA diameter: 5.4 cm) were treated with OPEN surgery (group A) and 22 (mean age: 74.1 years) (mean AAA diameter: 5.1 cm) were treated with EVAR. In 17 of them, there were no signs of endoleak (group B1), while in 5 patients, a presence of endoleak (group B2) was detected. Plasma samples were collected in order to determine MMP-9 activity. Enzyme immunoassay was performed preoperatively at 1, 3, 6 and 12 months. Patients treated conventionally were clinically examined after 1 and 12 months by ultrasound. Patients
undergoing EVAR treatment were clinically examined by CT scan after 1, 3, 6 and 12 months. The analysis was done by assessing the interaction over time of the MMP-9 value in B1 and B2 groups.

Results. — The average values observed for MMP-9 were preoperatively and at 1, 3, 6 and 12 months, respectively: in group A 150.8 ng/mL (SD = 30.5), 252.5 ng/mL (SD = 25.2), 315.4 ng/mL (SD = 22.7), 295.3 ng/mL (SD = 26.8), 210.7 ng/mL (SD = 30.2); in group B1 105 ng/mL (SD = 10.8), 125.6 ng/mL (SD = 19.9), 95 ng/mL (SD = 20.2), 80.4 ng/mL (SD = 15.6); in group B2 149 ng/mL (29.2), 375.4 ng/mL (SD = 40.2), 215 ng/mL (SD = 35.9), 180 ng/mL (SD = 20.2), 175 ng/mL (SD = 33.4). The MMP-9 level was higher in group B2 compared to group B1 (P = 0.01), suggesting a correlation with the presence of the endoleak.

Conclusions. — This preliminary study shows that MMP-9 may be a biomarker of the presence of endoleak. Other further investigations and larger series are needed to show that metalloproteases could play a role in the follow-up of EVAR treated patients.

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Introduction

The endoprosthesis treatment of abdominal aortic aneurysm (AAA) is now a well-known method which has seen extensive development, especially because of the good immediate results obtained. However, its long-term effectiveness, when compared to that of traditional surgery, has not been well defined yet, and the uncertainties which exist concerning its anatomical limits and the results over a period of time have prevented the indications for this type of treatment from being extended. Endovascular therapy is currently recommended for the elderly and more recently young patients with significant comorbidities [1–4]. The need to carefully monitor patients with an aortic endoprosthesis must be

MOTS CLÉS
AAA ;
Chirurgie ouverte ;
EVAR ;
MMP-9 ;
Suivi postopératoire ;
Biomarqueur
considered indispensable in order to detect early on any possible complications and carry out the most suitable therapy. In the past, in the largest analysis of the EUROSTAR Registry covering 1190 patients with a first-generation endoprosthesis who were followed for 8 years, surgical conversion was performed in 7.1% [5]. More recently, Jones et al. experienced a significantly higher rate of aneurysm rupture in patients with persistent type II endoleak (those that do not resolve within 6 months) compared with those without endoleak. From 0.6%–4.3% of the aneurysm ruptures are caused by type II endoleaks [3]. The need for long-term confirmation of the good results obtained immediately and in a medium-term period makes it necessary to continue to follow-up these patients at a considerable expense. In a study carried out on the follow-up, analysing data of 194 patients who received respectively EVAR (54) and OPEN (140) treatment, Hayter showed that it cost $18,000 to monitor one patient only for 2 years and that afterwards there was no need for additional expenditures, compared to the OPEN treatment [6]. Even though the results appear better in patients in whom second- and third-generation prostheses were implanted, it is necessary to bear in mind that in patients who received first-generation prostheses, the percentage from rupture/conversion is 48% at 5 years [5,7—9]. Although computerized tomography is considered the most common, reliable, and accurate method of surveillance, it must also be noted that it is necessary to repeat an examination several times. This implies two factors:

- exposing the subject to radiations which may induce a potential risk of carcinogenesis [10,11];
- administering a potentially nephrotoxic contrast medium [12,13].

In addition to instrumental diagnostic methods by imaging, recent studies have also suggested evaluating laboratory parameters which can be indicative of the risk of rupture due to the presence of endoleaks. On the basis of evidence which has emphasized the importance, among these parameters, of proteolytic enzymes in the genesis and growth of aneurysms, increasing interest has recently emerged concerning the significance of metalloprotease levels [14,15]. The existence of a correlation between the plasma level of metalloproteases (MMP-3 MMP-9) and the growth of abdominal aortic aneurysms has already been shown. Some authors have reported an initial increase in patients who have already received an endoprosthesis, which lasts up to the third postoperative month [16,17]. Sangiorgi, on the other hand, observed a continual decrease in enzyme activity, compared to the basal level of MMP-9, without any increase in the early phases after EVAR [18], while Hovsepian et al. noticed a decrease in the concentration of metalloproteases up to 91% in an average follow-up of 7 months [19]. Any increase in metalloprotease plasma levels during the surveillance period after endovascular exclusion of the aneurysmal sac could represent a marker in case of persistent endoleak or endotension.

The objective of this prospective clinico-experimental study is to evaluate the prognostic value of variations in the concentration of metalloproteases at the one-year follow-up of patients in whom either a conventional surgical operation or an endovascular one were performed for AAA.

### Materials and methods

Between November 2004 and July 2008, 255 operations were performed for AAA in the department of vascular surgery of the university of Rome Tor Vergata. Of this total, 202 patients (79%) received OPEN treatment and 53 (21%) EVAR treatment. The inclusion criteria for EVAR treatment are based on high risk patient: ASA (American Society Anesthesiology) III and IV category (patients with severe comorbidities such as heart disease, diabetes, respiratory and kidney failure) or obstile abdomen, with ideal morphology for EVAR treatment. The plasma level of MMP-9 was assayed in 33 patients of the OPEN group (group A) and 22 patients of the EVAR group (group B). The patients treated with the conventional technique were followed-up after 1 and 12 months with a clinical examination and echography, while those treated endovascularly were monitored with clinical and echographical examinations and CT after 1, 3 and 6 months, and then annually. Five of them presented signs of type II endoleak (group B1), with enlargement of AAA from 0.4 to 1 cm, while no signs of endoleak were detected in 16 without enlargement of the aneurysm sac (group B2). Cases of type I, III, or IV endoleak or signs of endotension were not observed.

Blood was sampled 1 day before surgery and 1, 3, and 6 months after the operation. The blood sample, obtained from a vein, was placed in test tubes with EDTA, centrifuged (2000 g for 15 minutes), separated, and kept at —80°C. The MMP-9 levels were determined with a commercially available kit of sandwich enzyme-correlated immunoadsorbent, which uses monoclonal antibodies (Matrix metalloproteinase-9, human, ELISA system, Amersham Pharmacia Biotech®), that are directed at different epitopes of MMP-9. The percentages of inaccuracy (intraseries < 6%, interseries < 10%) and the absence of cross-reactivity with proMMP-1, proMMP-2, proMMP-3, TIMP-1 and TIMP-2 (Tissue inhibitor of Metalloproinases 1—2) were determined by the manufacturer.

Statistical analysis reported data were analyzed by calculating the mean value. Where appropriate, differences in frequency were assessed by calculation of P value. In this case, P values summarize crucial evidence needed to gauge the error rates of measurements based on statistical tests. The analysis was done by assessing the interaction over time of the MMP-9 value in groups B1 and B2.

### Results

In group A, the average transverse diameter of the aneurysm was 5.4 cm (average age: 70.1). The average diameter was 5.1 cm in group B (average age: 74.1). No statistically significant differences were observed in the preoperative dimensions of the aneurysm between the surgical group (group A: average transverse diameter: 5.4 cm, 32 males, average age: 70.1 years) and the endovascular one (group B: average transverse diameter: 5.1 cm, 21 males, average age: 74.1).
The group of patients who received endovascular treatment differed from the group in whom conventional surgery was performed only in a higher ASA score or level of SVS/ISCVS of the Medical Risk Factor Categorization.

The baseline level of MMP-9 showed no significant differences between the two groups (group A: 150.8 ng/mL, group B: 127.1 ng/mL; *P* value = n.s.) (Table 1).

In the follow-up period considered, no deaths occurred either in the group in which the patients received OPEN treatment (group A) or in the EVAR treatment patients (group B). No complications related to the endovascular procedure were observed. After insertion of the endoprosthesis, 16 patients showed no signs of endoleak (group B1), while the presence of endoleak was detected in 5, at 30 days postoperative, associated with the growth of sac diameter from 0 to 1 cm (group B2). At one, 3, 6, 12 months following the procedure, no statistically significant differences were found in the average values of MMP-9 between the patients who received OPEN surgical treatment and those treated endovascularly, with and without endoleak (Table 2). In the light of our results, the patients who received endovascular surgery of aneurysmal exclusion, without endoleaks, presented an average decrease in the concentration of MMP-9 at 3 months compared with the preoperative values, while no significant reduction in the plasma levels was observed in the patients with endoleaks. Furthermore, a progressive tendency to an increase in the levels of MMP-9 was apparent at the 1-month follow-up; it was greater after conventional surgery and persisted up to 3 months, compared with what was observed following the endovascular procedure in the absence of endoleaks.

**Discussion**

This study underlines the potential strategic role of MMP. In particularly, high plasma levels of MMP-9 represent an important biological marker which is indicative of the presence of an AAA and that the variations of enzyme secretion represent a potential biomarker in EVAR therapy.

**MMP-9 and OPEN surgery**

It is possible to speculate that the significant increase in the average plasma levels of MMP-9 at 1 month, which persists up to 3 months after the conventional surgical treatment, is secondary to an acute inflammatory response or to an acute episode of a chronic inflammatory process, caused by the aspecific stress of major surgery or by manipulation of the aneurysmal sac during resection of the aneurysm. Moreover, the stress, secondary to conventional surgical treatment, may be sufficient to induce and amplify an acute inflammatory response which, by recruiting leukocytes, tends to stimulate regeneration of the damaged tissue and maintains itself with a further increase in the production of MMP-9 by the macrophage cells [20]. The traumatism generated on the tissues by the greater impact of the surgical act, compared with the femoral artery approach used for the endoluminal introduction of the endoprosthesis, induces an early perioperative response which causes a rise in the inflammatory response. The incision of the peritoneum, the traction of the mesenteric vessels, the loss of blood, the clamping of the aorta, the ischemia-reperfusion syndrome, the manipulation of the aortic sac, the synthetic material of the implanted prosthesis as well as the general anesthesia are the main events able to induce the inflammatory response and modulate its duration and intensity [21]. The increase of MMP-9 after surgery is reported [22].

**MMP-9 and EVAR surgery**

The progressive decrease in the concentrations of MMP-9, 6 months after the endovascular procedure, which is characterized by less invasiveness and limited surgical trauma, such as to elicit a less intense inflammatory response, may be interpreted as an expression of the complete and persistent exclusion of the aneurysmal sac. If, however, the levels of MMP-9 continue to be high, this may be indicative of the persistent blood flow within the aneurysmal sac which was previously excluded by the insertion of the endoprosthesis. In confirmation of this hypothesis, the titer of MMP-9 appears to fall, without returning to preoperative levels, after reduction of the endoleak.

In our study, we found that the group of patients who received endovascular treatment differed from the patients in whom classical surgery was performed only in a higher ASA score or level of SVS/ISCVS. Histomorphological studies, carried out as far back as 1980 by Busuttil, have shown that aneurysmal tissue is characterized by an increase in the collagenolytic and elastolytic

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**Table 1** Preoperative average plasma values of MMP-9.

<table>
<thead>
<tr>
<th>Group</th>
<th>Basal values (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>150.8</td>
</tr>
<tr>
<td>B</td>
<td>127.1</td>
</tr>
</tbody>
</table>

**Table 2** Postoperative average plasma values of MMP-9.

<table>
<thead>
<tr>
<th>Group</th>
<th>At 1 month (ng/mL)</th>
<th>At 3 months (ng/mL)</th>
<th>At 6 months (ng/mL)</th>
<th>At 12 months (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>252.5</td>
<td>315.4</td>
<td>295.3</td>
<td>210.7</td>
</tr>
<tr>
<td>B1</td>
<td>125.6</td>
<td>85.8</td>
<td>95</td>
<td>80.4</td>
</tr>
<tr>
<td>B2</td>
<td>375.4</td>
<td>215</td>
<td>180</td>
<td>175</td>
</tr>
</tbody>
</table>

activity, caused by an excessive production and activation of metalloprotease, compared with nonpathological aortic tissue [22]. It is now known that the progressive degradation of the fibrillar component of the extracellular matrix of the tunica media, caused by an inflammatory cellular infiltrate of the wall, is responsible for the development, progressive growth and potential rupture of AAAs [23]. McMillan and Pearce showed that high concentrations of soluble MMP-9 can be measured in the plasma of patients with AAA [24] by using the ELISA technique.

The possibility was therefore evaluated by following the trend of the MMP-9 plasma level after conventional and endovascular surgical treatment, compared with the baseline values observed during the perioperative period [16,18]. At present, the literature does not contain unequivocal reports, and the consequent implications from a prognostic point of view are not yet known. Moreover, until now, no study has compared the circulating levels of MMP-9 before and after conventional or endovascular surgical exclusion of the aneurysm, more than 6 months later.

The data which we observed differ from what Sangiorgi reported. He showed that both in conventional surgical exclusion and in endovascular treatment of the aneurysm, a significant decrease is seen in the plasma levels of MMP-9 compared with the preoperative baseline values, which is progressively greater at 1, 3 and 6 months [18]. Similar results were confirmed by Hovsepian, who found a 91% reduction in the enzyme activity of MMP-9 during an average follow-up of 7 months [19]. The reduction in the activity of MMP-9 in this series could be interpreted as secondary to the operating technique inasmuch as, once the aneurysmal sac has been opened and flattened, it is devitalized, with a drastic reduction in the inflammatory cellular infiltrate of the wall and induction of a process of cicatrization with an outcome of fibrosis, until the prosthesis has been completely incorporated.

A significant rise of MMP-9, in the short-term period after laparotomy, brings to mind the observations reported by Swanson in 1980 [25] and by Durham in 1991 [26], who noticed that aneurysms which are not diagnosed at the time of a laparotomic incision, performed for another pathology and not correlated with AAA, evolve rapidly with a consequent possible rupture. This phenomenon could explain the rapid rise in the levels of MMP-9, which was observed after 1 week by Taurino et al.; it was greater in the conventional surgery group (59.7 ± 16.8 ne/mL versus 41.7 ± 19.1 ne/mL) compared with the endovascular group (49.3 ± 32.4 ne/mL versus 44.4 ± 24.6 ne/mL). In the same series, it was also found that, at the 1-month follow-up, the levels of MMP-9 decrease in both groups but not significantly; with the exception of smokers in whom, compared with non-smokers, the preoperative serum concentration of MMP-9 was also greater, probably as a consequence of more serious wall damage in the former [17].

From what has been said so far, it may be deduced that the increase in MMP-9, after conventional surgery, does not assume a negative prognostic value and is an expression of the normal process of postoperative cicatrization. It may be speculated that a mechanism similarly correlated with enzyme mediators could be responsible for a high rate of growth, with the risk of rupture in a short period of time. In fact, in small aneurysms, in addition to the other morphological situations characteristic of this particular population of patients, high levels of MMP-9 could be considered as a risk factor which suggests a possible rupture.

In the case of the endovascular procedure, we observed a different behavior in the levels of concentration of MMP-9, depending on the presence or absence of endoleaks. In particular, the persistence after 1 month of the baseline values, with endoleaks, does not assume a negative prognostic value, inasmuch as it is presumably due to the peculiarity of the endovascular procedure: the insertion of the endograft within the aneurysmal vessel allows the exclusion of the aneurysmal sac from systemic circulation. However, even when it is performed successfully, it leaves the aortic wall unaltered. The macrophage cellular component, present in the inflammatory infiltrate, continues to secrete MMP-9 up to 3 months, when a rapid decrease occurs. The inflammatory response induced by the procedure, called post-implantation syndrome, could also be involved in this "phenomenon" [27,28].

The prognostic value that can be attributed to the significant increase in the plasma concentrations of MMP-9 is different; they present a peak which is reached at 1 month and a gradual decrease at 6 months, down to levels which, however, remain higher than those observed before the treatment (Fig. 1). It is important to consider the preoperative value as a landmark. This datum, which in our study refers to type II endoleaks, is confirmed by other authors and interpreted unequivocally as indicative of persistent endoleak [16—18]. Confirming this observation, Sangiorgi also showed that, after correction of the type I endoleak, the plasma level of MMP-9 had significantly reduced, returning to the values observed during the 6 pretreatment months; thus suggesting the complete exclusion of the aneurysmal sac.

At present time, there is no sufficient data in the literature which might identify other factors able to justify this evidence. Nonetheless, it may be speculated that other mediators of inflammation are involved in the process of stabilization or growth of the aneurysmal sac and that, in the event of perfusion of the sac after insertion of the endoprostheses, the equilibrium among these factors is altered.

An endoleak, the presence of a persistent flow of blood between the lumen of the aortic endoprosthesis and the interior of the aneurysmal sac, is still described as the "main complication of endovascular surgery". In these cases, an increase may occur in the size of the aneurysmal sac and in the intraluminal pressure, thereby augmenting the risk of rupture, even though there is still no unanimous agreement on whether and how to treat this clinical situation [29—31]. The incidence of this complication ranges between 0 and 47%, with an average of 20% (7% of which occurs in the first month and 13% in a later phase, 90 days after the operation), and critically depends on the type of endoprostheses used, the selection of the patient, the implantation technique, and the aortic morphology [7,9].

The potential role of MMP-9 in monitoring possible occurrence of endoleaks assumes considerable importance if one bears in mind that it may be of help in trying to predict the need for secondary surgical conversion (which is necessary in 0.7—3.7% of the cases [31,32]) or prevent the risk of rupture of the aneurysm. Actually, the AneuRx multicenter trial showed that the absence or presence of endoleaks at
the CT examination, done before discharging the patient, does not appear to be able to predict his survival [33], most likely because the majority of the perioperative endoleaks are corrected spontaneously during the first few months and need no further treatment [34,35]. The sac enlargement in the following period is also an important factor: as Bastos Gonçalves and colleagues report clinical success was highly dependent on sac growth and the generation of the implanted device influence sac growth and the original design grafts increase the risk of sac growth significantly [36]. Also in the Kaouel experience, the conversion after 18 months was necessary [37].

Therefore, it is important to monitor the instability of the aneurysmal sac, which would seem to be correlated with the trend of the plasma levels of MMP-9, especially in the presence of type I and III endoleaks which are associated with a greater risk of rupture equal to 3.37% in an average period of time of 15.4 months. Moreover, significant correlations have been found between an increase in the size of the aneurysm, noticed during follow-up, and the presence of endoleak at 2 years [5]. Heikkinen et al. showed that an endoleak is associated with an increase in the size of an aneurysm, but it is not correlated with a greater risk of rupture compared to patients without endoleak [33].

The importance of this phenomenon was emphasized by Rutherford, who points out the need for an assiduous, continual monitoring of patients who undergo EVAR; considering the analysis of the EUROSTAR Registry on 2463 patients, he reports an increase in the diameter > 8 mm in 15% of the patients with type II endoleak and the need for a second procedure [1]. This evaluation could further confirm the prognostic role of MMP-9 in cases of endotension, which cannot be observed with other instrumental diagnostic examinations, as they are not associated with detectable endoleaks.

Weyer et al. support that the follow-up of patients who received surgery of endovascular exclusion of the aneurysmal sac cannot be limited to evaluating only the maximum diameter of the vessel [38]; it has now been shown that this parameter is not sufficiently sensitive or specific for identifying conditions of supplying the sac and, above all, it presents serious limits in revealing cases of endotension. This occurrence cannot be detected with current diagnostic techniques, but it often becomes evident with an invasive assessment of the intrasac pressure or only after surgical conversion, or it might simply remain unknown even on the operating table [7].

The immunoenzymatic assay of MMP-9 could be useful in identifying the conditions of pressurization of the aneurysmal sac (which is not endoleak-correlated) in which high pressure is maintained inside the vessel and transmitted through the thrombotic apposition of the wall thus making it impossible to detect the escape route of blood flow to the outside of the endoprosthesis [19]. It is possible to speculate that in these cases, the increase in MMP-9 might be secondary to persistent secretion and enzymatic activity of the wall macrophages of the aneurysmal sac, induced by the persistence of pressure inside the vessel, which apparently does not encounter volumetric reduction. These remarks allow postulating that a reduction in the circulating MMP-9 levels, after endovascular treatment, represents a marker of the successful exclusion of the sac.

It may be speculated that the plasma assay of MMP-9 plays a potential role as a marker in the follow-up of patients who have received surgical or endovascular treatment; if necessary, the assay can be supplemented by an ultra-sonographic examination with echo-amplifying agents [39,40]. In fact, the spiral angio-CT presents diagnostic limits in identifying conditions of endopressure, even with the aid of coronal and sagittal axial multi-planar two-dimensional algorithms of reconstruction (MPR) and in particular with techniques of maximum intensity of projection (MIP) and volume rendering (VR) [35,37]. In a study carried out on a follow-up three years after insertion of an aortic stent-graft, Bendick compared the angio-CT examination to the ultra-sonographic examination with echo-amplifying agents and showed their sensitivity and specificity in monitoring complications, but less so for morbidity [41]. Other authors have also stressed their possible advantages [42,43].

The use of MMP-9 as a biomarker could reduce the need for checkups involving an angio-CT, thereby improving patient compliance. The assessment of MMP-9 during a

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**Figure 1** Histogram of the average plasma values of MMP-9 in patients treated for AAA. Group A: conventional surgery; Group B1: endovascular surgery without endoleaks; Group B2: endovascular surgery with endoleaks; Group B1—B2 difference: P value = 0.01.

**Histogramme des valeurs plasmatiques moyennes de MMP-9 chez les patients traités pour AAA. Groupe chirurgie conventionnelle A ; groupe chirurgie endovasculaire B1 sans endofuites ; groupe chirurgie endovasculaire B2 avec endofuites ; groupe B1/B2 différence : p = 0.01 évolution du taux au cours du temps.**
follow-up, as a potential alternative to instrumental examinations, could also contribute to reducing the total costs of the endovascular procedure. It is clear that endovascular treatment continues to require a long follow-up period, also because it is indispensable to establish whether the cost/benefit ratio critically depends on the combined reduction of long-term mortality and morbidity.

Conclusions

From what has been set forth, it may therefore be stated that in the follow-up of patients who received surgery of endovascular exclusion of the aneurysmal sac it could be useful to verify the role of the postoperative increase in MMP-9 as a predictive factor of rupture, especially when associated with a greater understanding of the phenomenon of endoleak or endotension. During long-term follow-up, metalloproteases could allow identifying those conditions of type II endoleak and, if present, of endotension which may expose the patient to a risk of rupture or require another procedure. The assay of MMP-9 may therefore take on particular importance in the presence of conditions of constant and persistent pressurization of the sac, which would otherwise remain unknown inasmuch as they cannot be detected with an angi-CT examination.

The prognostic value attributable to the assay of MMP-9, postulated in our study, needs to be confirmed by using a larger sample of patients. The method can be validated only by comparing data obtained in randomized prospective studies. Therefore, the immunoenzymatic evaluation of MMP-9, characterized by less invasiveness and lower costs compared with diagnostic imaging, in a complementary manner with the ultra-sonographic examination, could represent a potential aid in verifying the long-term success of the procedure. It is possible to speculate that metalloproteases may be considered an adequate aid to be used during follow-up, representing a biochemical marker of therapeutic success in the endovascular treatment of aneurysms of the abdominal aorta.

Disclosure of interest

The authors declare that they have no competing interest.

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