CLINICAL INVESTIGATION



Role and Effectiveness of Percutaneous Arterial Embolization in Hemodynamically Unstable Patients with Ruptured Splanchnic Artery Pseudoaneurysms

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Abstract

Purpose To assess the role and effectiveness of percutaneous arterial embolization (TAE) in patients with hemodynamic instability due to hypovolemic shock secondary to ruptured splanchnic artery pseudoaneurysms (SAPA).

Materials and Methods Seventeen patients (11 men, 6 women; mean age, 53 years) with hemodynamic instability (systolic blood pressure <90 mmHg) due to hypovolemic shock secondary to ruptured SAPA were treated by TAE. Clinical files, multidetector row computed tomography angiography, and angiographic examinations along with procedure details were reviewed.

Results Seventeen SAPAs were present, predominantly located on gastroduodenal or pancreatic arteries (9/17; 53 %). Angiography showed extravasation of contrast

medium from SAPA in 15/17 patients (88 %). Technical success rate of TAE was 100 %. TAE was performed using metallic coils in all patients (100 %), in association with gelatin sponge in 5/17 patients (29 %). TAE allowed controlling the bleeding and returning to normal hemodynamic status in 16/17 patients (94 %). In 1/17 patient (6 %), surgery was needed to definitively control the bleeding. The mortality and morbidity rate of TAE at 30 days were 0 and 12 %, respectively. Morbidity consisted in coil migration in 1/17 patient (6 %) and transient serum liver enzyme elevation in 1/17 patient (6 %).

Conclusion TAE is an effective and safe treatment option for ruptured SAPA in hemodynamically unstable patients, with a success rate of 94 %. Our results suggest that TAE should be the favored option in patients with hemodynamic instability due to ruptured SAPA.

Splanchnic artery pseudoaneurysms are safely and effectively treated by arterial embolization in hemodynamically unstable patients.

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Department of Gastroenterology and Endoscopy, Hôpital Lariboisière, Assistance Publique-Hôpitaux de Paris, 2 rue Ambroise Paré, 75010 Paris, France **Keywords** Arterial embolization · Pseudoaneurysm · Embolization · Hemorrhagic shock · Splanchnic arteries

Introduction

Pseudoaneurysm (or false-aneurysm) corresponds to a focal disruption of the arterial or venous wall [1, 2]. Histologically, arterial pseudoaneurysms are different from true aneurysms because they do not have the three layers of the arterial wall and contain either the media or adventitia or a loose connective tissue layer [1, 3]. They are therefore more fragile and have a tendency to enlarge progressively and to rupture [3].

Pseudoaneurysms can occur on a variety of arteries [1, 4–7]. Regarding splanchnic arteries, pseudoaneurysms have various causes; many of them are secondary to abdominal trauma or are a complication of pancreatitis resulting from destruction of the vessel wall by pancreatic enzymes [7–9]. More rarely, splanchnic artery pseudoaneurysms (SAPAs) may be secondary to intraoperative injury, tumors, pseudocysts, or peptic ulcer [8–10].

Emergency endovascular treatment is a well-established therapeutic for controlling ruptured aneurysms of splanchnic arteries, and it is currently assumed that this is also true in the specific case of ruptured SAPA [8]. So far, several studies have reported multiple cases of ruptured pseudoaneurysms causing gastrointestinal bleeding or hemoperitoneum [8, 10–18]. In spite of myriad single case reports of ruptured pseudoaneurysms in various locations causing severe bleeding and hemodynamic instability with a favorable outcome after percutaneous arterial embolization (TAE) [6, 19-22], to date in an emergency setting, open surgical repair of visceral artery pseudoaneurysms remains the gold standard. This is because data from the literature support the strong assumption that endovascular treatment is related to a non-negligible mortality when performed in an emergency setting [12]. However, studies

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have found that endovascular treatment may be considered as a reasonable option in the elective setting of hemodynamically unstable patients with ruptured SAPA [5, 12].

Accordingly, the goal of our study was to assess the role and effectiveness of TAE in patients with hemodynamic instability due to hypovolemic shock secondary to ruptured SAPAs.

Materials and Methods

Patients

A retrospective cohort study was performed to identify and analyze all patients with hemodynamic instability due to hypovolemic shock in association with ruptured SAPA, who were treated by TAE at our institution during the period from July 1994 to December 2012 inclusively. Hemodynamic instability due to hypovolemic shock was defined as a systolic blood pressure <90 mmHg, a fluid resuscitation requirement >2,000 mL or a blood transfusion >4 units within 24 h [23]. Our Review Board approved the retrospective data analysis. The need for informed consent was waived.

The final cohort comprised 17 patients with hemodynamic instability due to hypovolemic shock, who underwent TAE of splanchnic arteries for the treatment of a ruptured SAPA. There were 11 men and 6 women with a mean age of 53.2 years \pm 17.4 (SD) (range, 31–84 years). All medical records of these patients (including clinical, radiological, and surgical files, as well as discharge summaries) were reviewed using a standardized data collection form that included quantitative and qualitative variables. Among the 17 patients, 6/17 (35 %) were referred to us from outside institutions for emergency abdominal angiography and TAE. For these latter patients, the mean time from presentation at referring institution and time to TAE, including transfer time was 6 h and 40 min (range, 5-13 h). Among these six patients, three were originally stable at the referring institution, with a mean transfer time of 8 h (range, 5–13 h) and three were originally unstable at the referring institution with a mean transfer time of 5 h and 20 min (range, 5-6 h).

All patients had multidetector row computed tomography (MDCT)-angiography before TAE, either in our institution (11/17; 65 %) or at the referring institution (6/ 17; 35 %), so that the diagnosis of SAPA was initially made on MDCT-angiography and further confirmed during angiography. The diagnosis of SAPA was made in the presence of a well delineated, round or oval collection of contrast medium communicating with the parent artery lumen through a narrow neck on MDCT-angiography (Fig. 1), thus excluding direct bleeding with hematoma formation. No patients were excluded due to missing data.

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Fig. 1 67-year-old woman with hematemesis and hypovolemic shock 17 days after endoscopic duodenal stent placement (patient 6). A Multidetector row computed tomography (MDCT)-angiography

in the axial plane reveals well-delineated pseudoaneurysm (*arrow*). **B** MDCT-angiography in the oblique plane confirms arterial pseudoaneurysm (*arrow*) originating from hepatic artery (*arrowhead*)

All patients (17/17; 100 %) were hemodynamically instable and received packed red blood cells (mean = 5.1 units \pm 4.4 (SD); range: 2–10), fresh-frozen plasma (mean = 3.2 units \pm 2.9 (SD); range: 2–16), and crystalloid or colloid substances. The main clinical presentation was either intraperitoneal hemorrhage (10/17; 59 %) or acute gastrointestinal bleeding (7/17; 41 %). Demographic data of the 17 patients, presenting symptoms and causes of SAPA, are detailed in Table 1. Among the 17 patients, six (6/17; 35 %) had SAPA due to intraoperative arterial injury, with a mean time between surgery and TAE of 9.5 days (range, 2–24 days).

The mean hemoglobin level before TAE was 6.2 g/ dL \pm 1.4 (SD) (range: 3.2–8.2 g/dL). The mean systolic blood pressure at presentation was 75 mmHg \pm 8 (SD) (range; 62–85 mmHg). Disseminated intravascular coagulation that needed platelets transfusion alone or in association with coagulation factors administration (recombinant coagulation Factor VIIa, NovoSeven[®], Novo Nordisk Inc., Plainsboro, NJ, USA; fibrinogen, Clottafact[®], LFB Biomedicaments, Courtaboeuf, France) was present in 6/17 patients (35 %) before TAE.

Angiographic Technique

Abdominal angiography was performed using a femoral approach with a 5-Fr catheter (Cobra Radifocus[®], Terumo Medical Corporation, Tokyo, Japan) and a 0.032-inch guidewire (Radifocus[®], Terumo Medical Corporation). Selective catheterization of the splanchnic artery that was considered as the origin of the pseudoaneurysm on the basis of global angiogram was first performed. Special attention was given to the presence, if any, of extravasation of contrast medium. Selective catheterization of the

feeding arteries was attempted in all patients using digital road mapping. Frontal projections were obtained in all patients, and oblique projections were performed when needed. Angiograms were performed using non-ionic iodinated contrast medium that was injected by hand.

After TAE, control angiogram was performed to ensure complete occlusion of SAPA and patency of parent artery. All patients were continuously monitored for hemodynamic status by an intensive care physician during the procedure. They further had close surveillance in the intensive care unit of our institution during the following 12 h.

Data Collection

For all patients, qualitative and quantitative variables were tabulated using a standardized data collection form. Original angiograms and interventional radiology reports were reviewed in consensus by two radiologists with extensive experience in TAE. Angiograms were analyzed using the workstation of a picture archiving and communication system (PACS) (Directview[®], V 11.4, Carestream Health Inc, Rochester, NY, USA). For each patient, dimensions, location, feeding arteries of SAPA, and if any contrast extravasation were recorded. The location of the SAPA with respect to the feeding artery was categorized following a well-established anatomy [24, 25]. Details of each procedure were recorded after reviewing the interventional radiology data sheets, including details regarding occluding agents used.

TAE was initially analyzed in terms of technical success and technical failure depending on a successful or a failed catheterization of parent artery, satisfactory or unsatisfactory coil deployment, and exclusion or persisting

Patient no.	Age	Gender	Presenting symptoms	Cause of SAPA	Hb level (g/ dL)	Delay (days)
1	45	Male	Upper gastrointestinal bleeding (hematemesis)	Involvement by gallbladder cancer	3.2	N.A.
2	84	Female	Upper gastrointestinal bleeding (hematemesis)	Acute pancreatitis	5.5	N.A.
3	54	Male	Upper gastrointestinal bleeding (hematemesis)	Involvement by pancreatic adenocarcinoma	4.4	N.A.
4	45	Male	Upper gastrointestinal bleeding (hematemesis and wirsungorrhagia)			N.A.
5	35	Male	Hemoperitoneum	Intraoperative injury (cholecystectomy)	7.0	6
6	67	Female	Upper gastrointestinal bleeding (hematemesis)	Duodenal stent migration	5.6	N.A.
7	32	Male	Hemoperitoneum	Pancreatic pseudocyst/Chronic pancreatitis	6.1	N.A.
3	46	Male	Hemoperitoneum	Acute pancreatitis	6.0	N.A.
)	62	Male	Hemoperitoneum	Acute pancreatitis	6.2	N.A.
10	31	Female	Hemoperitoneum	Injury during percutaneous renal biopsy	7.5	2
11	78	Male	Hemoperitoneum	Intraoperative injury (duodenopancreatectomy)	6.8	24
12	61	Female	Hemoperitoneum	Intraoperative injury (partial hepatectomy)	6.0	13
13	37	Male	Upper gastrointestinal bleeding (hematemesis and wirsungorrhagia)	Pancreatic pseudocyst/Chronic pancreatitis	6.1	N.A.
14	83	Female	Hemoperitoneum	Intraoperative injury (duodenopancreatectomy)	7.8	10
15	58	Male	Hemoperitoneum	Acute pancreatitis	7.0	N.A.
16	36	Female	Upper gastrointestinal bleeding (hematemesis and hemobilia)	Intraoperative injury (cholecystectomy)	8.2	4
17	50	Male	Hemoperitoneum	Intraoperative injury (small-bowel resection)	7.9	8

Table 1 Clinical data of 17 patients with hemodynamic shock and ruptured splanchnic artery pseudoaneurysm

All patients presented with hemodynamic instability and hemorrhagic shock

SAPA indicates splanchnic artery pseudoaneurysm. Delay indicates the time interval (in days) between the causative event (i.e., intraoperative injury) and percutaneous transarterial embolization

N.A. indicates not applicable

opacification of SAPA. TAE was considered as successful when the bleeding resolved without the need for surgery. Failure of TAE was defined as a bleeding that needed urgent surgery in spite of a second TAE session or even after a first TAE session only when hemodynamic status or clinical symptoms indicated urgent surgery.

Associated morbidity and mortality \leq 30 days were also documented by reviewing clinical files and discharge summaries.

Statistical Analysis

Descriptive statistics were calculated for the clinical variables and those evaluated at angiography. For quantitative data they included means, standard deviations, and ranges. For qualitative data descriptive statistics included raw numbers, proportions, and percentages.

Results

Seventeen TAE procedures were performed. Successful catheterization of parent arteries, satisfactory coil deployment, and exclusion of SAPA on control angiogram were achieved in all procedures, yielding a technical success rate of 100 % (95 % CI: 80–100 %).

Seventeen SAPAs were present on angiographic studies. Locations of SAPAs included predominantly gastroduodenal or superior pancreaticoduodenal arteries (n = 9; 53 %), followed by superior mesenteric artery (n = 2; 12 %), hepatic artery (n = 2; 12 %), and cystic artery stump (n = 2; 12 %) (Fig. 2). The locations of SAPAs are given in details in Table 2. Angiography showed contrast extravasation from SAPA in 15/17 angiograms (88 %). No extravasation was present in 2/17 examinations (12 %) (Fig. 2). Pushable metallic coils (SPI 2x25, SPI 2x50, SPI 3x50, Balt, Montmorency, France or MicroNester[®] MWCE 18-14-8; Cook Medical, Bloomington, IN) were used in all patients (17/17; 100 %). They were introduced under fluoroscopic guidance through a 2.8-Fr (RenegadeTM, Boston Scientific, Natick, MA) or 2.7-Fr (ProgreatTM, Terumo Medical Corporation) microcatheter used coaxially. TAE was performed with a mean number of 5.2 metallic coils \pm 2.7 (SD) per patient (range: 2–14 coils).

Pledgets of gelatin sponge (Gelitaspon[®], Gelita Medical BV, Amsterdam, The Netherlands) measuring $1 \times 1 \times 20 \text{ mm}^3$, with a total number comprised between 4 and 9, were used in combination with metallic coils in 5/17 patients (29 %).

Control angiogram showed complete exclusion of SAPA and no extravasation after all procedures. The parent artery was occluded after TAE in 13/17 procedures (76 %) and continued to opacify in 4/17 procedures (24 %). Sac embolization only was performed in 4/17 procedures (24 %), parent artery occlusion with inflow/outflow vessel occlusion was performed in 1/17 procedure (6 %), and sac embolization along with parent artery occlusion with inflow/outflow vessel occlusion was performed in 12/17 procedures (70 %). No newly developed vessels giving blood supply to the SAPA were observed in either case on control angiograms.

Clinical symptoms disappeared and hemodynamical status returned to normal in 16/17 patients (94 %) during or less than 2 h after a single TAE procedure, yielding a clinical success rate of 94 % (95 % CI: 71–100 %). In 1/17 patient (6 %), no improvement in hemodynamical status was observed after TAE; in this latter patient repeated TAE was not considered because of a progressive worsening in hemodynamic status. This patient (patient 4) had emergency surgery that consisted in duodenopancreatectomy and ligation of gastroduodenal artery.

No complications due to the TAE procedure were noted in 15/17 patients (88 %) whereas complications related to TAE were observed in 2/17 patients (12 %), resulting in a morbidity rate of 12 %. In 1/17 patient (6 %), coil migration occurred during TAE but was followed by successful retrieval using the Lasso loop technique (Patient 10). In 1/17 patient (6 %), TAE was followed by a transient increase in serum level of liver enzymes that resolved spontaneously in less than 10 days (Patient 16) with a return to normal values.

Except for patient 16, no patients had clinical symptoms or biological abnormalities that suggested distal organ ischemia in relation to TAE. No deaths related to TAE occurred during the 30 days following TAE (0 % mortality rate). No patients experienced recurring bleeding during the 30 days following TAE.

Discussion

We report the experience of a single-center study that included 17 patients with ruptured SAPAs that were responsible for hemodynamic instability due to hypovolemic shock. Our results showed that ruptured SAPAs are appropriately treated by TAE with a success rate of 94 %. Only one patient required further surgery for stopping the bleeding because of failed TAE. The rate of technical success for TAE was 100 %, with a mortality rate of 0 % and a morbidity rate of 12 %. Our results are consistent with those reported in a prior study with less case material [18]. Loffroy et al. have reported a series of 12 patients with SAPA, who were treated successfully by endovascular coil embolization [18].

In our study, we observed one failed TAE in a patient with ruptured pseudoaneurysm of the gastroduodenal artery due to pancreatic pseudocyst. In the study by Udd et al. that included 33 patients with bleeding pancreatic pseudoaneurysms and pseudocysts treated by TAE, the success rate of TAE was 67 % (22/33 patients) [13]. Of interest, in 10 patients, the bleeding could not be stopped either because of technical failure (7 cases) or inability to access the bleeding vessel (3 cases), so that surgery was required [13]. Similarly, in the study by Marshall et al. including five patients with pancreatic artery pseudoaneurysm treated by TAE, surgery was needed in four of them, thus suggesting that this location may be the least favorable one for TAE [10].

The analysis of data from the literature reveals that pseudoaneurysms responsible for gastrointestinal bleeding or hemoperitoneum are mostly located on the splenic artery [18]. In the study by Boudghene et al., pseudoaneurysms due to acute pancreatitis were more predominantly located (42 %) on the splenic artery [7]. In the study by Vimalraj et al., pseudoaneurysms arose from the splenic artery in 17/26 patients, the gastroduodenal artery in 5/26 patients, a pancreaticoduodenal artery in 3/26 patients, and from the superior mesenteric artery in 1/26 patient [26]. By contrast, in our series, no cases of splenic pseudoaneurysm were observed.

The diagnosis of SAPA can be made using various imaging tests [9, 24, 27–29]. In our study, the diagnosis of SAPA was initially made using MDCT-angiography and further confirmed at angiography in all patients. Some authors advocate the use of MDCT-angiography to confirm the presence of and localize pseudoaneurysm [1]. They found that CT angiography helped demonstrate the location of the pseudoaneurysm and identify feeding vessels owing to three-dimensional reformatted images [1]. In addition, angiography reveals luminal characteristics with limited information about the wall that MDCT-angiography would provide, including the presence of thrombus and other

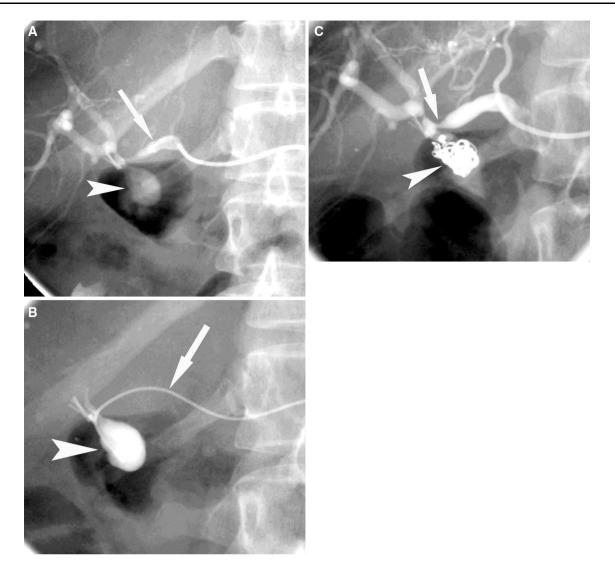


Fig. 2 36-year-old woman with hypovolemic shock 4 days after laparoscopic cholecystectomy suggesting intraperitoneal hemorrhage due to intraoperative vascular injury and possibly pseudoaneurysm (patient 16). A Angiogram of the right branch of hepatic artery (*arrow*) shows pseudoaneurysm originating from cystic artery stump (*white arrow*). B Selective angiogram with a 2.7-Fr microcatheter (*arrow*) placed into the pseudoaneurysm (*arrowhead*) shows no

tissues that have organized to form the wall or the sac of the pseudoaneurysm [29].

On angiogram, pseudoaneurysm presents as a focal collection of iodinated contrast material that communicates with the parent artery lumen usually through a narrow neck. There is an accumulation of contrast material in the perivascular space that disappears slowly. In our series, no extravasation of contrast medium from SAPA was present on 2/17 angiograms although the corresponding patients were actively bleeding. In these two patients, TAE was performed, resulting in hemodynamic stability and no more bleeding. Similar scenarii have been reported in the

extravasation of contrast material and allows precise measurement of pseudoaneurysm dimensions. C Selective angiogram after arterial embolization of pseudoaneurysm with metallic coils shows complete filling of pseudoaneurysm (*arrowhead*) by metallic coils, without persisting intraluminal opacification of pseudoaneurysm and satisfactory opacification of parent artery (*arrow*)

literature so far [22]. It is commonly admitted that bleeding may be intermittent and a patient with clinical signs of ongoing blood loss may have no visible active bleeding at the time of angiography. Our results suggest that in patients with SAPA, TAE must be performed even in the absence of active bleeding from SAPA on angiographic examination.

In general, patients with ruptured SAPA present with intraperitoneal bleeding. More rarely, they may present with gastrointestinal bleeding when the SAPA bleeds in the Wirsung duct and when the blood escapes into the gastrointestinal tract from the papilla of Vater. This occurrence corresponds to the so-called hemosuccus

Patient no.	Location of pseudoaneurysm	Diameter (mm)	Embolized segments	Embolization agent (number of coils)	
1	Right replaced hepatic artery	5	Parent artery	Metallic coils (14)	
				Gelatin sponge pledgets	
2	Superior mesenteric artery	15	SAPA	Metallic coils (8)	
3	Celiac trunk	22	SAPA	Metallic coils (5)	
4	Superior pancreatic artery	22	SAPA + parent artery + gastroduodenal artery	Metallic coils (5)	
5	Cystic artery stump	15	SAPA + parent artery	Metallic coils(8)	
				Gelatin sponge pledgets	
6	Hepatic artery	15	SAPA + parent artery	Metallic coils (3)	
7	Superior pancreatic artery	47	SAPA + parent artery	Metallic coils (6)	
8	Superior pancreatic artery	9	SAPA + parent artery + gastroduodenal artery	Metallic coils (3)	
				Gelatin sponge pledgets	
9	Superior pancreatic artery	15	SAPA + parent artery + gastroduodenal artery	Metallic coils (3)	
				Gelatin sponge pledgets	
10	Left colic artery	12	SAPA + parent artery	Metallic coils (4)	
11	Gastroduodenal artery	8	SAPA + parent artery	Metallic coils (2)	
12	Gastroduodenal artery	16	SAPA + parent artery	Metallic coils (3)	
13	Gastroduodenal artery	10	SAPA + parent artery	Metallic coils (3)	
				Gelatin sponge pledgets	
14	Gastroduodenal artery	17	SAPA + parent artery	Metallic coils (5)	
15	Gastroduodenal artery	14	SAPA + parent artery	Metallic coils (5)	
16	Cystic artery stump	16	SAPA	Metallic coils (6)	
17	Superior mesenteric artery	25	SAPA	Metallic coils (6)	

 Table 2
 Angiographics findings and endovascular treatment characteristics in 17 patients with hemodynamic shock due to ruptured splanchnic artery pseudoaneurysm

All patients presented with hemodynamic instability and hemorrhagic shock. SAPA indicates splanchnic artery pseudoaneurysm

pancreaticus, which is relatively rare and almost uniformly due to a pseudoaneurysm of the splenic artery [26]. The efficacy of TAE in hemosuccus pancreaticus has been reported as low as 50 % [26]. Our series include two cases of hemosuccus pancreaticus. Of note, the single patient of our series, who had an unfavorable outcome after TAE and who needed further surgery to stop the bleeding presented with this condition.

Analysis of the existing literature shows that several occluding agents can be used for TAE in patients with SAPA, including metallic coils [17, 18, 30], *n*-butyl-2-cyanoacrylate [14, 29], or a combination of two different materials [12, 31]. Others have reported the use of covered stents [32]. The use of metallic coils has been reported in several studies and many interventional radiologists favor their use for the treatment of SAPA because it is assumed that they do not carry the potential risk of arterial rupture due to high pressure injection of gelatin sponge pledgets [18]. Another reason is that they can be delivered directly into the pseudoaneurysm sac without compromising the patency of the parent artery. Conversely, the use and deployment of covered stent may be limited by marked tortuosity of the parent vessel [18].

Some authors advocate the use of sac embolization with coils (i.e., the packing technique) for the treatment of pseudoaneurysms [18]. Loffroy et al. have suggested that this technique is safe and effective and may result in lower morbidity than parent artery occlusion (i.e., inflow/outflow vessel occlusion) because it preserves parent artery patency, thus maintaining perfusion of distal organ [18]. However, parent artery occlusion of both sides of pseudoaneurysm remains the most commonly used technique. One limitation of the exclusive use of the packing technique is that it can be performed only in patients with actual pseudoaneurysm that has an actual sac to pack, so that the diagnosis of pseudoaneurysm must be ascertained by MDCT-angiography prior to TAE.

Our study has several limitations. The first is due to the retrospective nature of the study and the fact that in our institution TAE is the favored option for patients with active bleeding so that no comparison with surgery could be possible in retrospect. Second, resuscitation was performed by a panel of anesthesiologists with an extensive experience in the management of patients with severe bleeding and hypovolemic shock so that our results in terms of feasibility and mortality may not be transposable to other institutions with a more limited experience. Third, a subset of our patients were treated relatively early after the intraoperative traumatic event so that it may be argued that for these patients the bleeding lesion may not be an actual pseudoaneurysm according to the histopathological definition.

In conclusion, the results of our series reinforce the general assumption that TAE is an effective and safe treatment option for ruptured SAPAs that cause hemodynamic instability and hemorrhagic shock. In addition, TAE is associated with a low morbidity. Our results suggest that TAE should be the favored option in patients with hemodynamic instability due to ruptured SAPA although further comparative studies with more case material should be made to fully confirm this assumption.

Conflict of interest Anthony Dohan, Clarisse Eveno, Raphael Dautry, Youcef Guerrache, Marine Camus, Mourad Boudiaf, Etienne Gayat, Olivier Le Dref, Marc Sirol, Philippe Soyer declare that they have no conflicts of interest.

Statement of Informed Consent "Informed consent was obtained from all individual participants included in the study."

Statement of Human Rights "All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards."

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