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### ► To cite this version:

Pierre-Olivier Comby, Kévin Guillen, Olivier Chevallier, Emilie Couloumy, Anne Dencausse, et al.. Blocked-flow vs. free-flow cyanoacrylate glue embolization: Histological differences in an in vivo rabbit renal artery model. *Diagnostic and Interventional Imaging*, 2024, 105 (4), pp.137-143. 10.1016/j.diii.2023.10.003 . hal-04804749

**HAL Id: hal-04804749**

**<https://hal.science/hal-04804749v1>**

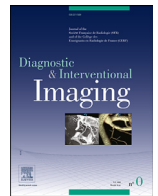
Submitted on 2 Dec 2024

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Original article

## Blocked-flow vs. free-flow cyanoacrylate glue embolization: Histological differences in an in vivo rabbit renal artery model

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### ARTICLE INFO

#### Keywords:

Animal model  
Blocked-flow injection  
Cyanoacrylate glue  
Free-flow injection  
Transcatheter embolization

### ABSTRACT

**Purpose:** The purpose of this *in vivo* animal study was to compare the acute histological effects on the arterial vessel wall of free-flow vs. blocked-flow embolization with metacryloxysulfolane-n-butyl cyanoacrylate (MS-NBCA) in several concentrations.

**Materials and methods:** A total of 42 rabbit renal arteries were embolized using MS-NBCA mixed with ethiodized oil. The MS-NBCA concentration was 12.5%, 25%, or 50%. All mixtures were injected under both free-flow and blocked-flow conditions. The rabbits were euthanised 30 min after arterial embolization. Arterial-lumen distension, intimal inflammation and necrosis, peri-arterial edema, and distality of MS-NBCA penetration were assessed histologically. Multivariable regression analyses were performed using a manual backward procedure, with linear, ordinal and logistic regression to search for factors associated with these outcomes

**Results:** Marked or severe dilatation was observed in 36 out of 42 arteries (86%) and marked or transmural intimal arteritis in all 42 arteries (42/42; 100%). Lumen dilatation caused focal vessel-wall flattening, which resulted in intimal necrosis. Multifocal necrosis extending from the intima to the media occurred in 23 out of 42 kidneys (55%) and peri-arterial edema with multifocal vascular leakage in 19 out of 42 kidneys (45%). At multivariable analysis, blocked-flow MS-NBCA injection was associated with greater severity of vessel-wall lesions, including intimal arteritis ( $P = 0.003$ ) and intimal necrosis ( $P = 0.014$ ), compared to free-flow injection. Blocked-flow injection was also associated with peri-arterial edema ( $P = 0.008$ ) and greater distality of MS-NBCA penetration ( $P = 0.001$ ).

**Conclusion:** Blocked-flow MS-NBCA injection during renal artery embolization is significantly associated with more acute arterial-wall damage and greater distality of glue penetration compared to free-flow injection in a rabbit model. These preliminary findings may have clinical implications, as blocked-flow injection is routinely used to treat specific vascular diseases or malformations in human.

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### 1. Introduction

Cyanoacrylate glue is used as an embolic agent for several decades [1]. The nearly instantaneous and permanent target-vessel occlusion is a major advantage but can also be challenging. The utilization of cyanoacrylate glue spans across a multitude of embolization indications,

encompassing its application in both arteries and veins [1]. Within the venous system, the primary indications include the management of pelvic congestion syndrome and varicoceles through gonadal vein embolization, pre-hepatectomy portal vein embolization, and the treatment of chronic venous insufficiency via embolization of lower limb veins. In arterial contexts, its primary applications are centered around the management of hemorrhage, treatment of arteriovenous malformations, and intervention in tumoral lesions. Tumor embolization with glue addresses several potential objectives, including the alleviation of pain, inhibition of tumor growth, induction of size reduction, and management of acute hemorrhage. It often serves as a

**Abbreviations:** AE, Absolute ethanol; MS-NBCA, Metacryloxysulfolane-n-butyl cyanoacrylate; NBCA, n-butyl cyanoacrylate; SD, Standard deviation

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<https://doi.org/10.1016/j.diii.2023.10.003>

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Please cite this article as: P.-O. Comby, K. Guillen, O. Chevallier et al., Blocked-flow vs. free-flow cyanoacrylate glue embolization: Histological differences in an in vivo rabbit renal artery model, *Diagnostic and Interventional Imaging* (2023), <https://doi.org/10.1016/j.diii.2023.10.003>

preparatory step for surgery by minimizing blood loss and facilitating the resection process [1–3]. Intra-arterial glue distribution must be accurately controlled during the injection to ensure that the occlusion is confined to the target vessel, preventing reflux into the parent vessel and the occurrence of unintended emboli. Cyanoacrylate glues are radiolucent and solidify rapidly by polymerisation upon contact with body fluids. They are therefore injected in combination with ethiodized oil (Lipiodol® Ultra Fluid, Guerbet, Villepinte, France), which not only provides radiopacity but also slows the polymerisation rate. The glue concentration can be chosen to obtain the polymerisation rate that best suits the treatment goal [4]. A lower glue concentration results in greater distality of glue penetration within the vascular bed [5]. Absolute ethanol (AE) can also be added to the mixture to accelerate polymerisation and change the adhesive properties of the glue [6].

The pressure cooker technique was initially developed for non-adhesive liquid embolic agents containing ethylene vinyl alcohol copolymer dissolved in dimethyl sulfoxide [7,8]. However, recent studies have demonstrated its potential utility in adhesive glue embolization as well. This technique, by allowing proximal vessel occlusion, decreases the risk of reflux along the microcatheter and enhances distal glue progression within the vascular network. [9]. Regardless of how occlusion is obtained (*i.e.*, with a double-lumen balloon or proximal glue plug with coils), this technique stops blood flow around the microcatheter during glue injection, thereby modulating the polymerisation reaction.

The tissue toxicity of cyanoacrylate glue has been extensively studied [10–15]. Glue deposition within a vessel triggers an acute inflammatory response in the vessel wall and surrounding tissues. A foreign-body reaction with giant cells and fibrosis is apparent after approximately 30 days. N-butyl-2 cyanoacrylate (NBCA), which is the most widely used cyanoacrylate glue, can be combined with metacryloxysulfolane (MS), resulting in metacryloxysulfolane-n-butyl-2 cyanoacrylate (MS-NCBA, Glubran®2, GEM) to lessen the cytotoxic and inflammatory effects [16]. Histopathological studies have focused on lesions produced by cyanoacrylate glue injection under free-flow conditions. However, blocked-flow injection is now used in clinical practice in several indications.

The objective of this study was to investigate acute histological arterial vessel-wall changes after embolization with MS-NBCA in several concentrations under both free-flow and blocked-flow conditions, in an *in vivo* animal model.

## 2. Materials and methods

### 2.1. Animal model and embolization procedure

The local animal-experimentation ethics committee approved the study protocol (CEEA n°106, #25592 approved 23/06/2020). The animals were housed and studied in a laboratory accredited by the French Ministry of Health for research on animal models. Requirements set forth in the 2019 updated version of the European Directive 2010/63/EU were followed. Twenty-six female rabbits purchased from Charles River (Écully, France) were used for this study.

The rabbits were sedated with intramuscular buprenorphine (Buprecare® 0.3 mg/mL, 0.17 mL/kg body weight; Animalcare) and anesthetised with 2% isoflurane (Zoetis). Two interventional radiologists (POC and KG) with seven and four years of experience, respectively, performed the procedures as described previously [17], using a monoplane angiographic system (Veradius, Philips.). After a surgical approach, a 4-French (Fr) introducer sheath was inserted through the femoral artery. A baseline angiogram of the abdominal aorta was obtained using a 4-Fr vertebral catheter by injecting manually a water-soluble contrast agent lobitridol (Xenetix® 350, Guerbet) using a 10 mL syringe with an estimated injection rate of approximately 4 mL/s. The renal arteries were catheterized consecutively by introducing a 2.3-Fr microcatheter (Phenom® 21, Medtronic) coaxially

through the 4-Fr catheter and positioning it at a distance of one centimeter before the initial bifurcation of the renal artery. Special attention was given not to be selective in the ventral or dorsal branch. Blocked-flow conditions were obtained by introducing the 4-Fr vertebral catheter into the proximal renal artery to completely stop the blood flow, as confirmed by the stagnation of contrast agent injected gently into the microcatheter. The placement of the distal tip of the 4-Fr vertebral catheter in the proximal part of the renal artery was sufficient to achieve blocked-flow conditions. The external diameter of the 4-Fr catheter measures 1.78 mm. Ultrasound measurements of the diameter of the main renal artery in rabbits were conducted during our feasibility study and found a diameter of approximately 1.5 mm. Furthermore, the placement of the distal end of the 4-Fr catheter resulted in an angulation of the artery, contributing to vessel occlusion. The occlusion was confirmed by injecting contrast through the microcatheter. The contrast was promptly washed out upon catheter removal, which was then repositioned before glue injection. No spasms were observed during any of the procedures. Every renal artery was embolized at its proximal portion. The 4-Fr vertebral catheter was moved temporarily to the aorta to allow contrast elimination then repositioned. Free-flow conditions were achieved by leaving the 4-Fr vertebral catheter in the aorta. Washout into the kidney parenchyma of contrast injected selectively through the microcatheter was documented to confirm proper catheter positioning.

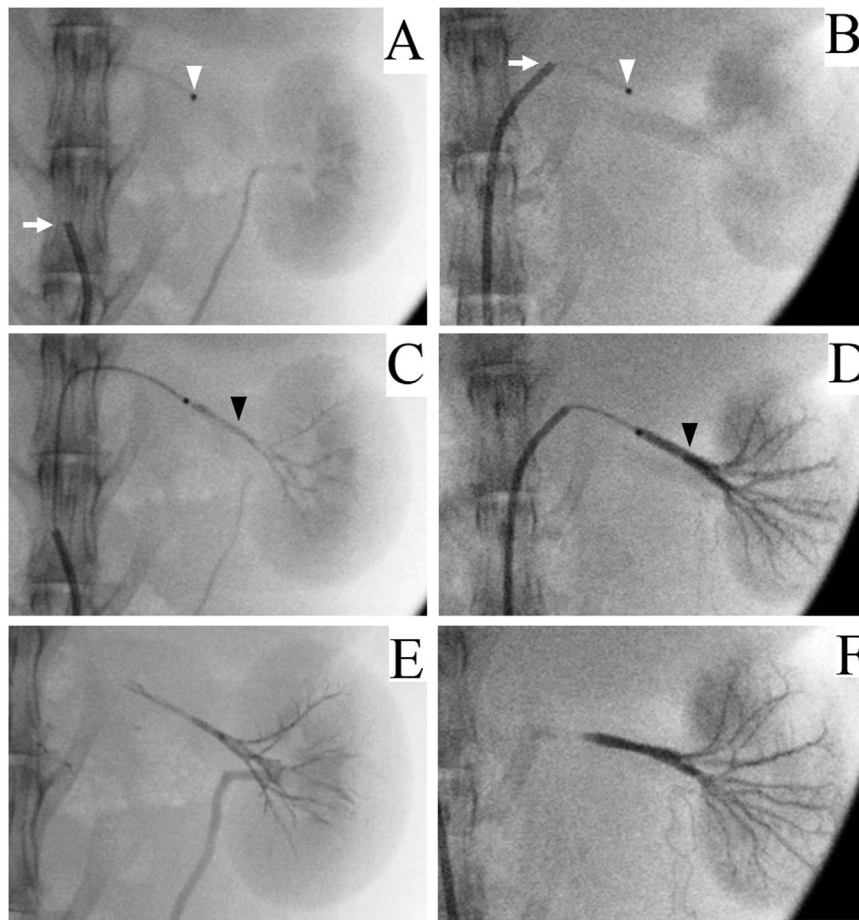
Before glue injection, the microcatheter was flushed with 5% dextrose to prevent premature glue polymerisation. The amount of dextrose injected prior to glue injection was at least 0.43 mL, which corresponded to the dead space of the microcatheter. The mixture was injected under fluoroscopic monitoring using a 5-mL syringe (Plastipak, Becton Dickinson Plastic) and an automatic syringe pump (Harvard Apparatus PHD 2000), allowing a standardized injection rate of 0.03 mL/s. This flow rate was adjusted to the physical characteristics of the rabbit model, particularly the vessel diameter, based on our clinical experience with embolization using NBCA. Embolization was considered complete when reflux along the microcatheter was identified. The microcatheter was then immediately removed to prevent tip adhesion to the vessel wall. The inner lumen of the 4-Fr vertebral catheter was aspirated, and the contralateral renal artery was embolized using a new microcatheter. The injected volume was measured by subtracting the luminal volume of the microcatheter (0.43 mL) from the total volume injected. Injection duration was reported for each procedure. Animals were euthanized, under general anesthesia, using an intravenous bolus of euthasol (0.34 mL/kg, Med-Vet International), administered 30 min after the completion of the last embolization procedure. The key steps of free-flow and blocked flow glue embolization are illustrated in Fig. 1.

### 2.2. Glue mixtures

MS-NBCA was mixed manually with ethiodized oil at room temperature immediately before the injection. Four mixtures were studied; three containing only MS-NBCA and ethiodized oil in ratios of 1:1, 1:3, and 1:7, respectively, and one containing MS-NBCA, ethiodized oil, and AE in a ratio of 1:2:1 (Table 1). The absolute glue concentrations were 50%, 25%, and 12.5%. All four mixtures were injected under free-flow and blocked-flow conditions, and each mixture was injected into five or six renal arteries.

### 2.3. Histopathological evaluation

Each kidney-renal artery unit was collected and immediately fixed in 10% neutral-buffered formalin solution. The formalin-fixed samples were trimmed and paraffin-embedded. Longitudinal sections of each kidney at two to four levels were prepared and stained with hemalun-eosin. The resulting 121 histological slides were assessed by a veterinary pathologist (T.L.) blinded to sample data.



**Fig. 1.** Fluoroscopic images show left renal artery embolization with metacryloxysulfolane-n-butyl cyanoacrylate at a concentration of 25% under free-flow and blocked-flow conditions. A and B: Fluoroscopic image shows microcatheter positioning prior to embolization within the renal artery. In (A), the distal end of the 4-Fr vertebral catheter (arrow) extends into the aorta for free-flow embolization, while in (B), the distal end of the 4-Fr vertebral catheter (arrow) is placed within the proximal segment of the renal artery for blocked-flow embolization. Arrowheads indicate the distal tip of the microcatheter. C and D: Angiograms of renal arteries (arrowheads) obtained during injection of metacryloxysulfolane-n-butyl cyanoacrylate at a concentration of 25% under conditions of free-flow (C) and blocked flow (D). E and F: Angiograms show final cast distribution after microcatheter removal.

**Table 1**  
Experimental details on the 42 renal arteries.

Variables	Free-flow injection	Blocked-flow injection	<i>P</i> value
Number of embolized arteries	20 (20/42; 48%)	22 (22/42; 52%)	0.827
Rabbit weight (kg)	3.25 (3, 3.5) [2.53–4.38]	3.09 (2.79, 3.44) [2.53–3.83]	0.24
Injected glue-mixture volume (mL)	0.23 (0.2, 0.35) [0.21–0.35]	0.41 (0.29, 0.55) [0.23–1.52]	<b>0.003</b>
Injection duration (s)	23.1 (22.4, 25.1) [19.1–23.3]	29.4 (25.8, 33.9) [22.9–66.1]	<b>0.003</b>
NBCA-MS 12.5% concentration	5 (5/42; 12%)	6 (6/42; 14%)	> 0.999
NBCA-MS 25% concentration	5 (5/42; 12%)	6 (6/42; 14%)	> 0.999
NBCA-MS 50% concentration	5 (5/42; 12%)	5 (5/42; 12%)	> 0.999
NBCA-MS 25% concentration and anhydrous ethanol	5 (5/42; 12%)	5 (5/42; 12%)	> 0.999

Quantitative variables are reported as medians followed by interquartile ranges (Q1, Q3) in parentheses and ranges into brackets. Qualitative variables are expressed as raw numbers; numbers in parentheses are proportions followed by percentages. Others variables are reported as raw number; numbers in parentheses are percentages.

Bold indicates a significant *P* value.

Each individual tissue lesion was examined. Arterial lumen dilatation was evaluated using a 4-level scale (0, none; 1, mild; 2, moderate; and 3, severe). Intimal inflammation defined as the presence of inflammatory cells, primarily lymphocytes and monocytes, within the subendothelial compartment of one or more arteries was quantified using the Banff 97 scale with 0 indicating no arteritis, 1 mild intimal arteritis, 2 marked intimal arteritis with at least 25% of the luminal area lost, and 3 transmural arteritis and/or arterial fibrinoid change [18,19]. Intimal necrosis was rated as follows: 0, none; 1, focal intimal necrosis with smooth muscle vacuolisation; and 2, multifocal necrosis with distended and flattened intima. Peri-arterial edema was recorded as absent or present.

The distality of glue distribution was evaluated by dividing the renal arterial tree into five levels: 1, renal artery and proximal branches; 2, interlobar arteries; 3, cortico-medullary junction; 4, interlobular arteries of the deep cortex; and 5, interlobular arteries of the superficial cortex. The highest level on all sections for each kidney was recorded by the veterinary pathologist.

#### 2.4. Statistical analysis

In order to account for the non-normal distribution of certain variables, all quantitative data were presented using medians, interquartile ranges (Q1, Q3), and ranges [20]. The scores for lumen dilatation, inflammation, necrosis, and distality were ordinal variables and peri-arterial edema was a dichotomous variable.

Mean baseline values were compared using the chi-square test and Kruskal-Wallis test as appropriate. Multivariable regression analyses were performed using a manual backward procedure, with linear regression for continuous variables, ordinal regression for ordinal variables, and logistic regression for the dichotomous variable (*i.e.*, periarterial edema). The optimal model was built by constructing several intermediate models to confidently exclude potentially confounding variables, such as MS-NBCA concentration, rabbit weight, and injected glue volume. Values of  $P < 0.05$  with a confidence interval not encompassing 0 were considered significant. Statistical analysis was performed using STATA 3 software (StataCorp LP, College Station).

### 3. Results

#### 3.1. Experiments

Both renal arteries in all 26 rabbits were embolized; however, in five animals the second renal artery was embolized using a different liquid embolization agent and was excluded from the analysis. The total number of renal arteries included in the analysis was 42 (26 right and 16 left), including 22 renal arteries embolized using blocked-flow injection. The rabbits' age was 3 months, with a median weight of 3.1 kg (Q1, Q3: 2.94, 3.45; range: 2.64–4.38 kg). The median volume of injected glue and injection duration were notably greater in the blocked-flow group compared to the free-flow group ( $P = 0.003$ ). Table 1 reports details on the 42 procedures.

#### 3.2. Histological findings

##### 3.2.1. Microscopic cast features

The visual characteristics of the NBCA glue casts remained largely consistent across all four concentration mixtures. Typically, the glue

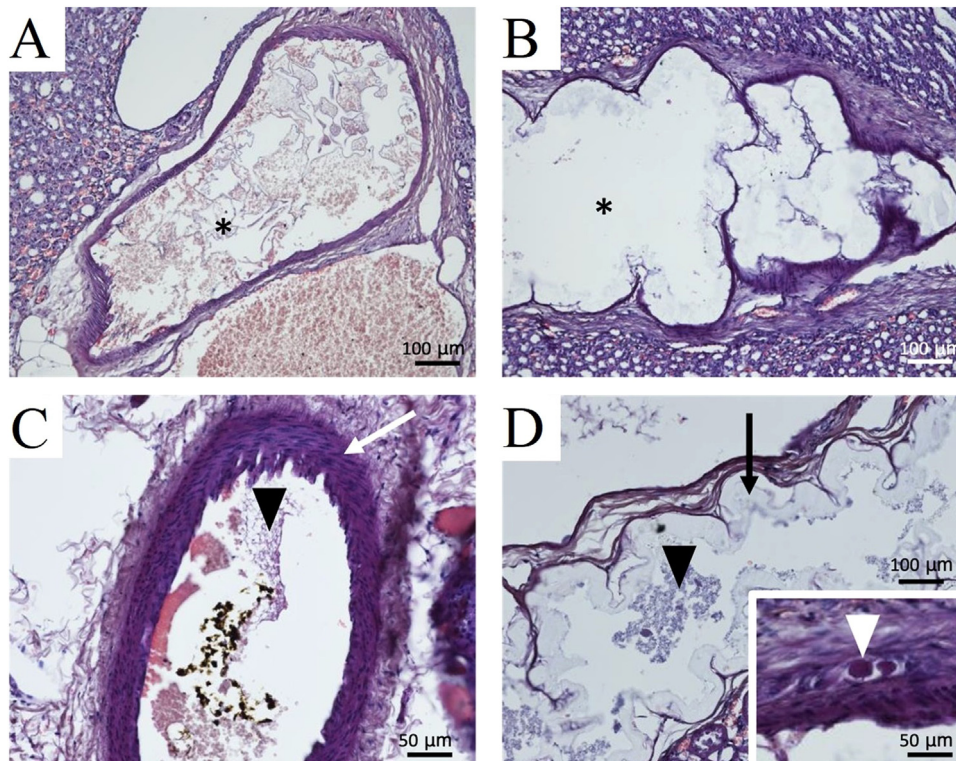
appeared as pale, refractive strands that adhered focally to the vessel wall and occasionally to the endothelium. In some instances, the glue exhibited a slightly basophilic or amphophilic foamy appearance, floating freely within the vessel lumina. The resulting casts displayed irregular plug-like structures, often interspersed with red blood cells and occasional fibrin thrombi. Crucially, there was no observation of glue extravasation outside the arterial lumens in any of the animals or tissue sections. The overall disruption of blood flow induced a widespread stasis effect in numerous tissue sections. These general findings are illustrated in Fig. 2.

##### 3.2.2. Arterial lumen and wall alterations

Lumen dilatation was seen in all 42 renal arteries and was severe in 36 (86%) kidneys. All 42 samples showed marked or transmural arteritis. In 28 out of 42 kidneys (67%), arterial-wall inflammation met criteria for the highest Banff 97 score of 3, indicating transmural arteritis. The severe luminal dilatation caused focal flattening of the vessel wall, which induced intimal necrosis in 39 out of 42 kidneys (93%). Multifocal necrosis extending from the intima to the media was observed in 23 out of 42 kidneys (55%). Peri-arterial edema with multifocal vascular leakage was present in 19 out of 42 kidneys (45%). Data are presented in Table 2. Histological findings are depicted in Fig. 2.

##### 3.2.3. Distribution of glue assessed through histological evaluation

In all 42 kidneys, glue was observed in the interlobular arteries in the deep or superficial cortex. The highest possible distality score of 5 indicating glue in the middle or distal portions of the interlobular arteries was noted in 32 out of 42 kidneys (76%). In the remaining 10 kidneys (24%), the distality score was 4, with glue in the arcuate arteries or proximal part of the interlobular arteries (Fig. 3).



**Fig. 2.** Photographs show histopathological findings after embolization of renal arteries with metacryloxysulfolane-n-butyl cyanoacrylate (hemalum-eosin stain). Glue (\*) within severely dilated arteries with flattened walls and partial (A) or total (B) occlusion of the lumen. The glue appears as foamy amphiphilic aggregates floating freely in the vessel lumen (C, black arrowhead) or as strands of refringent material (D, black arrow) lining the arterial wall and containing clusters of basophilic granular material (black arrowhead). The white arrow points to a region of transmural arteritis and the white arrowhead to severe arterial-wall necrosis and fibrinoid necrosis.

**Table 2**  
Histological findings in the 42 rabbit kidneys.

Findings	Free-flow injection	Blocked-flow injection	Total
<b>Arterial lumen dilatation</b>			
None	0 (0/20; 0%)	0 (0/22; 0%)	0 (0/42; 0%)
Mild	5 (5/20; 25%)	1 (1/22; 5%)	6 (6/42; 14%)
Marked	8 (8/20; 40%)	10 (10/22; 45%)	18 (18/42; 43%)
Severe	7 (7/20; 35%)	11 (11/22; 50%)	18 (18/42; 43%)
<b>Intimal arteritis</b>			
None	0 (0/20; 0%)	0 (0/22; 0%)	0 (0/42; 0%)
Mild	0 (0/20; 0%)	0 (0/22; 0%)	0 (0/42; 0%)
Marked	10 (10/20; 50%)	4 (4/22; 18%)	14 (14/42; 33%)
Transmural	10 (10/20; 50%)	18 (18/22; 82%)	28 (28/42; 67%)
<b>Intimal necrosis</b>			
None	3 (3/20; 15%)	0 (0/22; 0%)	3 (3/42; 7%)
Focal	10 (10/20; 50%)	6 (6/22; 27%)	16 (16/42; 38%)
Multifocal	7 (7/20; 35%)	16 (16/22; 73%)	23 (23/42; 55%)
<b>Peri-arterial edema</b>			
No	14 (14/20; 70%)	9 (9/22; 41%)	23 (23/42; 55%)
Yes	6 (6/20; 30%)	13 (13/22; 59%)	19 (19/42; 45%)
<b>Distality of glue penetration</b>			
Renal artery and first branches	0 (0/20; 0%)	0 (0/22; 0%)	0 (0/42; 0%)
Interlobar artery	0 (0/20; 0%)	0 (0/22; 0%)	0 (0/42; 0%)
Cortico-medullary junction	0 (0/20; 0%)	0 (0/22; 0%)	0 (0/42; 0%)
Interlobular arteries of the deep cortex	9 (9/20; 45%)	1 (1/22; 5%)	10 (10/42; 24%)
Interlobular arteries of the superficial cortex	11 (11/20; 55%)	21 (21/22; 95%)	32 (32/42; 76%)

All variables are expressed as raw numbers; numbers in parentheses are percentages.

### 3.2.4. Multivariable analysis

At multivariable analysis, blocked-flow injection was associated with worse vessel-wall damage, including significantly higher frequencies of intimal arteritis ( $P = 0.003$ ) and intimal necrosis ( $P = 0.014$ ), and with peri-arterial edema ( $P = 0.008$ ). Blocked-flow injection also produced more distal glue penetration ( $P = 0.001$ ). A larger injected glue volume was associated with peri-arterial edema ( $P = 0.026$ ) (Table 3).

Neither glue concentration nor addition of AE was associated with cast distality, arterial lumen dilatation, arterial-wall inflammation, or peri-arterial edema.

## 4. Discussion

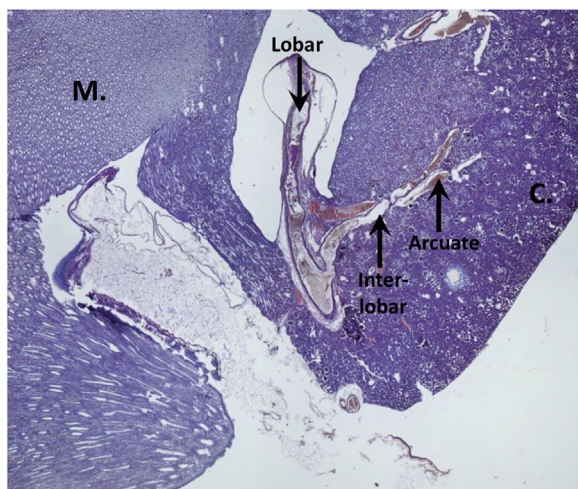
In our in vivo animal model, blocked-flow injection of cyanoacrylate glue to embolize the renal artery was significantly associated

with more vessel-wall damage compared to free-flow injection. Blocked-flow injection also resulted in the glue penetrating further distally along the vessel network. Injected glue volume was associated with peri-arterial edema but not with any of the other markers of vessel-wall damage. Neither glue concentration nor the use of AE was significantly associated with any of the histological findings.

Cyanoacrylate glue embolization is widely used to treat vascular malformations and tumours as a means of achieving rapid and permanent occlusion of the target vessels. Cyanoacrylate glues have toxic effects on tissues, although NBCA is less toxic than other derivatives such as bucrylate® or isobutyl 2-cyanoacrylate [21]. Inflammation, necrosis, and fibrosis have been reported. The underlying mechanism may be the release during glue polymerisation of both heat and toxic by-products such as formaldehyde [22]. The presence of residual monomers and degree of cross-linking within the polymer may also play a role.

One hypothesis explaining the more pronounced vessel-wall damage observed with blocked-flow injection is that the reduction in reflux led to a higher volume of injected glue. The multivariate analysis found no association linking glue volume to markers of vessel-wall damage other than peri-arterial edema, but the small sample size might explain this finding. Another hypothesis suggests that the elevated pressure exerted on the vessel walls due to both flow blockage and simultaneous glue injection could have played a role in causing vascular wall injuries. This potential mechanism is attributed to the mechanical effects stemming from wall distension. The fact that vessel damage was not influenced by glue concentration lends support to this hypothesis. However, in rat and rabbit models used to study free-flow embolization, tissue damage was not significantly different between low and high NBCA concentrations [14]. Blocked-flow NBCA injection is used in several indications, notably to decrease prostate volume or provide tumor devascularisation [23]. Studies to determine whether pain and local inflammation are more common in clinical practice after these procedures compared to free-flow glue embolization would be of interest. From another perspective, it can be speculated that the occurrence of increased vascular necrosis and tissue damage in the case of injection under blocked flow conditions could be considered an advantage, for instance in pre-surgical tumor embolization.

Understanding the beneficial and deleterious effects of blocked-flow glue injection is crucial. Studies with longer intervals from



**Fig. 3.** Photograph shows histopathological findings after free-flow embolization with metacryloxysulfolane-*n*-butyl cyanoacrylate at a concentration of 25% and its distribution along the arterial tree. The segmental, lobar, interlobar, and arcuate arteries are dilated and nearly completely occluded by glue aggregates. M indicates medulla; C indicates cortex.

**Table 3**

Multivariable ordinal or binary logistic regression analysis of factors associated with the arterial lumen dilatation, intimal inflammation, intimal necrosis, peri-arterial edema and cast distality.

Variable	B Coefficient (95 % CI)	P value
<b>Arterial lumen dilatation</b>		
Flow condition	1.18 (−0.24 to 2.60)	0.104
Volume injected	0.86 (−2.64 to 4.36)	0.630
Glue concentration	1.62 (−3.26 to 6.5)	0.515
Anhydrous ethanol	0.69 (−0.57 to 1.94)	0.285
Rabbit weight	1.3 (−0.14 to 3.07)	0.138
<b>Intimal inflammation</b>		
Flow condition	1.60 (1.15 to 3.04)	<b>0.003</b>
Volume injected	0.21 (−2.79 to 3.21)	0.890
Glue concentration	−1.03 (−6.53 to 4.46)	0.712
Anhydrous ethanol	0.20 (−1.44 to 1.84)	0.813
Rabbit weight	0.84 (−0.89 to 2.57)	0.341
<b>Intimal necrosis</b>		
Flow condition	1.64 (0.32 to 2.94)	<b>0.014</b>
Volume injected	−0.69 (−3.54 to 2.16)	0.636
Glue concentration	−3.06 (−8.48 to 2.34)	0.267
Anhydrous ethanol	−0.27 (−1.87 to 1.32)	0.741
Rabbit weight	0.85 (−0.38 to 2.09)	0.178
<b>Peri-arterial edema</b>		
Flow condition	2.38 (0.62 to 9.55)	<b>0.008</b>
Volume injected	5.09 (0.61 to 9.55)	<b>0.026</b>
Glue concentration	−3.44 (−8.7 to 1.83)	0.201
Anhydrous ethanol	−0.13 (−1.84 to 1.59)	0.886
Rabbit weight	0.96 (−0.88 to 2.54)	0.182
<b>Cast distality</b>		
Flow condition	2.73 (1.08 to 4.38)	<b>0.001</b>
Volume injected	−0.70 (−8.44 to 7.04)	0.860
Glue concentration	−1.08 (−6.00 to 5.30)	0.646
Ethanol	0.36 (−1.34 to 2.05)	0.682
Rabbit weight	−1.83 (−4.13 to 0.46)	0.170

CI indicates confidence interval. Bold indicates a significant P value.

embolization to histological assessment and with larger sample sizes are needed. The greater distality of glue penetration with blocked-flow injection may allow the successful embolization of targets not accessible under free-flow conditions [24]. Moreover, by enabling a longer injection duration before the occurrence of glue reflux along the microcatheter, blocked-flow injection allows better control of the glue cast and its progression within the vascular network.

The utilization of glue under blocked-flow conditions indeed holds considerable promise in enhancing control over glue behavior, facilitating deeper penetration of the embolic agent, and minimizing the risk of reflux. These techniques have become integral components of the interventional radiologist's toolkit. The primary aim of our study was to provide an objective account of the potential histological effects during the acute phase of such injection conditions. The extent of the inflammatory response can be viewed either as a drawback or an advantage, contingent upon the clinical context. Embolization of both benign and malignant lesions, particularly in a preoperative setting, may benefit from this approach by promoting either greater tumor destruction or immune system activation.

NBCA concentration was not associated with penetration distality in our study. In contrast, in the same rabbit model, lower NBCA concentrations were associated with greater distality of penetration as assessed using X-ray microtomography [17]. This cross-sectional method assesses the cast as a whole, instead of only on two to four sagittal slices as used in our study, and may therefore provide a more accurate evaluation of cast distality. Another option for histological studies is measurement of the smallest diameter of arterioles containing NBCA [5,25]. This method may be more objective than ours.

Although the kidneys in our study were assessed only 30 min after glue injection, they consistently demonstrated severe arterial-wall inflammation. In other studies, this finding was noted only after two to three days [13,14]. One possible explanation to this apparent

discrepancy is that we continued the injection until reflux occurred along the microcatheter, resulting in a larger injected glue volume. However, glue volume was not associated with vessel-wall damage in our study. Due to the terminal vascularisation of kidneys, ischaemic injury induced by the arterial obstruction may have contributed to the histological damage documented in our study. However, the short time from embolization to sacrifice minimised the risk of bias due to ischaemia.

Adding AE to NBCA-ethiodized oil may offer advantages such as faster polymerisation [24], less adhesion to the catheter [26], and less vessel-wall toxicity [27]. In our study, however, the presence of AE was not significantly associated with the histological findings. Possible explanations are the limited reproducibility of the AE mixtures and the small size of the AE groups. Moreover, differences related to the addition of AE might become apparent after a longer post-embolization interval.

The limitations of our study include the small number of embolization procedures with each of the four mixtures, related to material constraints and to our efforts to comply with the reduction component of the 3Rs principle. The use of an automatic injector deviated from standard clinical conditions. However, we opted for the use of an automatic injector in this study to achieve uniform injection procedures across the rabbits. This decision was deemed appropriate because our study primarily aimed to provide explanatory insights. The animals had healthy arteries. As mentioned above, the terminal vascularisation of the kidneys may have influenced our histological results. Finally, the differences between rabbits and humans regarding coagulation, vessel diameters, and other features of the vasculature must be considered when interpreting the findings.

In conclusion, our study demonstrates a significant association between MS-NBCA embolization using blocked-flow injection and increased vessel-wall damage during the acute phase. This finding may have clinical implications for embolization procedures employing the blocked-flow technique. In certain situations, the extent of the inflammatory response can be seen as an advantage, particularly for tumor or prostatic embolization, potentially enhancing tumor destruction. However, in other situations, it may be disadvantageous, such as in the embolization of superficial arteriovenous malformations, potentially leading to increased pain and local inflammation, necessitating the early introduction of analgesics or anti-inflammatory drugs. Further investigations with larger sample sizes and longer follow-up periods are warranted to provide a more comprehensive understanding of these effects.

#### Author contributions

All authors attest that they meet the current International Committee of Medical Journal Editors (ICMJE) criteria for Authorship.

#### Compliance with ethical standards

The local animal-experimentation ethics committee approved the study protocol (CEEA n°106, #25592 approved 23/06/2020). The animals were housed and studied in a laboratory accredited by the French Ministry of Health for research on animal models. Requirements set forth in the 2019 updated version of the European Directive 2010/63/EU for the protection of animals used for scientific purposes were followed.

#### Credit author statement

POC, KG, PR, SC, AVS and RL, conception and design of the study. POC, KG, EC and AD performed the experiments. POC, KG, OC performed data acquisition. POC, KG, OC, PR, AVS and RL interpreted the results. SAG conducted the statistical analysis. POC and RL drafted the

manuscript. All authors edited and revised the manuscript critically for important intellectual content. All authors read and approved the final version of the manuscript.

### Declaration of Competing Interest

The experiments were performed at the R&D Department of the Guerbet Corporation. The rabbits and Lipiodol Ultra Fluid® were founded by Guerbet (Villepinte, France). Guerbet had no role in data collection, analysis, or interpretation, manuscript drafting or revision. Emilie Couloumy, Anne Dencausse, Philippe Robert, Sarah Catoen are employees of Guerbet.

### Funding

This study was funded by R&D, Guerbet Research, 95943, Roissy Charles de Gaulle, France.

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