Selective angiographic embolization of blunt splenic traumatic injuries in adults decreases failure rate of nonoperative management

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BACKGROUND: To determine whether angioembolization (AE) in hemodynamically stable adult patients with blunt splenic trauma (BST) at high

risk for failure of nonoperative management (NOM) (contrast blush [CB] on computed tomography, high-grade IV–V injuries, or

decreasing hemoglobin) results in lower failure rates than reported.

METHODS: The records of patients with BST from July 2000 to December 2010 at a Level I trauma center were retrospectively reviewed using

National Trauma Registry of the American College of Surgeons. Failure of NOM (FNOM) occurred if splenic surgery was required

after attempted NOM. Logistic regression analysis was used to identify factors associated with FNOM.

RESULTS: A total of 1,039 patients with BST were found. Pediatric patients (age <17 years), those who died in the emergency department, and

those requiring immediate surgery for hemodynamic instability were excluded. Of the 539 (64% of all BST) hemodynamically stable patients who underwent NOM, 104 (19%) underwent AE and 435 (81%) were observed without AE (NO-AE). FNOM for the various groups were as follows: overall NOM (4%), NO-AE (4%), and AE (4%). There was no significant difference in FNOM for NO-AE versus AE for grades I to III: grade I (1% vs. 0%, p = 1), grade II (2% vs. 0%, p = 0.318), and grade III (5% vs. 0%, p = 0.562); however, a significant decrease in FNOM was noted with the addition of AE for grades IV to V: grade IV (23% vs. 3%, p = 0.04) and grade V (63% vs. 9%, p = 0.03). Statistically significant independent risk factors for FNOM were grade IV to V injuries and CB.

CONCLUSION: Application of strictly defined selection criteria for NOM and AE in patients with BST resulted in one of the lowest overall FNOM

rates (4%). Hemodynamically stable BST patients are candidates for NOM with selective AE for high-risk patients with grade IV to V injuries, CB on initial computed tomography, and/or decreasing hemoglobin levels. (*J Trauma*. 2012;72: 1127–1134.

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LEVEL OF

EVIDENCE: III, therapeutic study.

KEY WORDS: Blunt splenic trauma; angioembolization; contrast blush.

onoperative management (NOM) has become the standard of care for hemodynamically stable adult patients with low-grade (I–III) blunt splenic trauma (BST).^{1,2} However, multiple recent trials have reported failure of NOM (FNOM) rates approaching 67% to 100% with such factors as age >55, high-grade (IV–V) injuries, contrast blush (CB) on computed tomography (CT), large hemoperitonuem, and decreasing hemoglobin.^{3–5} Over the last decade, the selective application of angioembolization (AE) as an adjunct to the NOM of high-risk groups has resulted in reduction of the overall failure rates to as low as 2% to 4%.^{6–9} Initial postembolization complication concerns for splenic rupture or splenic abscess have also been minimal.^{10–12} Given the lack of randomized trials evaluating AE, its role and effectiveness in the management of BST remain to be defined. The purpose

of this study was to test the hypothesis that the addition of AE to standard NOM of hemodynamically stable adult patients with BST at high risk for FNOM (CB on initial CT, high-grade IV–V injuries on initial CT, and/or decreasing hemoglobin levels during NOM observation) results in lower failure rates than reported for NOM alone.

PATIENTS AND METHODS

The records of patients with BST over an 11-year period from July 1, 2000, to December 31, 2010, at a Level I trauma center were retrospectively reviewed using National Trauma Registry of the American College of Surgeons. Patients excluded from the original dataset included pediatric patients (age <17 years), patients who died in the trauma center, splenic injuries from penetrating trauma, and splenic injuries from iatrogenic intraoperative misadventures. Of the remaining patients, those who were found to be hemodynamically unstable after their initial evaluation in the trauma bay and transported directly to the operating room (OR) for abdominal exploration were deemed the operative management (OM) group and also excluded. The remaining adult (age ≥17 years) hemodynamically stable patients with BST were placed into the NOM study group. These patients were admitted to a monitored setting where hemodynamic parameters were followed and abdominal examinations and hemoglobin levels were checked in a serial fashion. Patients who developed peritonitis on abdominal examination and/or hemodynamic instability were taken immediately to the OR. Hemodynamically stable patients with decreasing hemoglobin levels were AE as a salvage maneuver. The level of decrease in hemoglobin used to trigger AE was dependent on attending surgeon judgment. For all patients, demographic information, grade of splenic injury, presence or absence of CB on initial CT, indications for AE, angiographic findings, and type of AE were all reviewed. Splenic injuries were

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graded according to the American Association Trauma Organ Injury Scale. 13

The National Institutes of Health (NIH) scoring system for hemodynamic instability as proposed by the Western Trauma Association (WTA) was used to objectively define initial patient hemodynamic status and for subsequent comparisons of NOM decisions. 14,15 This system has grades (I–V) of severity of hemodynamic compromise according to the systolic blood pressure (SBP) and response to volume resuscitation, with the highest grades (III–V) being most severe and requiring surgery for hemorrhage control.

Two groups of NOM patients were analyzed, those who underwent AE and those with no angioembolization (NO-AE). Splenic AE was performed during the study period only for one or more of the following indications: CB on initial CT, high-grade IV to V injuries on initial CT, and/or decreasing hemoglobin after admission during NOM observation. FNOM occurred if a patient required splenic surgery at any time after an attempt of NOM with or without AE. The University of Florida College of Medicine-Jacksonville Institutional Review board reviewed and approved the study protocol.

AE was performed in all cases using the following technique. The common femoral artery was accessed, and under fluoroscopic guidance, and a 5-French reverse curve Mickelson (Cook, Bloomington, IN) catheter was introduced, and the celiac and splenic arteries were selectively catheterized. Based on the pattern of active arterial bleeding and parenchymal blush identified patients had either a proximal main splenic artery embolization (PMSAE) or both PMSAE and selective distal splenic artery embolization (SDSAE) by using the microcatheter system to deploy multiple Tornado coils (Cook) of various sizes into the segmental or main branches of concern with follow-up images verifying positioning of the coils. The specific procedure in each patient

was at the discretion of the attending interventional radiologist. Follow-up imaging was not routinely done unless new symptoms or signs of problems developed.

This study included only the hospital course of patients until discharge. Although all patients were provided appointments for follow-up in our outpatient clinics, there are no data as to the completeness of follow-up nor long-term problems that may have developed.

Data are presented as the mean \pm SEM. Numerical variables were analyzed by the analysis of variance and categorical variables were analyzed by Fisher's exact test and χ^2 test. Logistic regression analysis was performed to evaluate the contribution of AE to successful NOM. A value of p < 0.05 was considered statistically significant.

RESULTS

There were 1,039 patients with BST identified from July 1, 2000, to December 31, 2010. The number of patients undergoing NOM gradually increased from 50% to 72% from 2000 to 2003 and then remained relatively stable at this level with minor fluctuations from 2004 to 2010. A similar but opposite trend was proportionally noted for the percent of patients who underwent OM over this time period (Fig. 1). One hundred two patients died shortly after arrival, 308 went directly to the OR for hemodynamic instability, and 90 were of pediatric age (age <17 years). These were all excluded from further analysis. The remaining 539 hemodynamically stable patients underwent NOM, NO-AE (435 patients), and AE (104 patients). Nineteen NO-AE patients failed NOM (4.4%) and four AE patients failed NOM (3.8%), for a total FNOM of 4.3% (23/539) (Fig. 2). Comparison of the demographic, clinical, and mechanism characteristics of these three groups (OM, NO-E, and AE) indicated only a higher Injury Severity Score (ISS) and mortality, and lower admission SBP

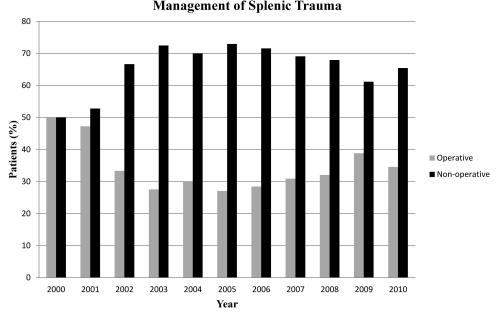


Figure 1. Trends in the operative and nonoperative management strategies over a decade from 2000 to 2010 for BST.

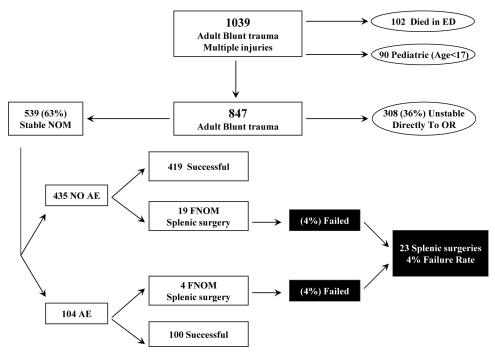


Figure 2. Flow chart of the management of 847 adult BST patients.

and discharge home rate, in OM versus NOM groups. Comparison of the two NOM groups (AE vs. NO-AE) indicated that the AE group contained patients with a significantly

TABLE 1. Demographic and Clinical Variables According to Treatment Group

		NOM	NOM	
	OM	NO-AE	AE	Statistical
Variable	(n = 308)	(n = 435)	(n = 104)	Significance
Male	197 (64%)	281 (65%)	75 (72%)	NS
Female	111 (36%)	154 (35%)	29 (28%)	NS
Age	39 ± 17	38 ± 17	37 ± 16	NS
ISS	30 ± 13	20 ± 12	26 ± 11	*†‡
Low grade (I-III)	_	401 (92%)	43 (41%)	‡
High grade (IV-V)	_	34 (8%)	61 (59%)	‡
Admission SBP	104 ± 33	124 ± 26	119 ± 20	*†
Admit to ICU	_	217 (50%)	65 (63%)	NS
Mortality	93 (30%)	32 (7.3%)	8 (8%)	*†
Discharged home	127 (41%)	314 (72%)	72 (69%)	*†
Mechanism of injury				
MVC	199 (65%)	271 (62%)	67 (64%)	NS
MCC	42 (13%)	56 (13%)	8 (8%)	NS
PVA	22 (7%)	25 (6%)	3 (3%)	NS
FALL	23 (7%)	42 (9%)	13 (12%)	NS
ATV	5 (2%)	8 (2%)	4 (4%)	NS
Other	17 (6%)	33 (8%)	9 (9%)	NS

MVC, motor vehicle crash; MCC, motorcycle crash; PVA, pedestrian vs. auto crash; ATV, all terrain vehicle; GCS, Glasgow Coma Scale; ICU, intensive care unit.

higher level of injury as demonstrated by the higher ISS and greater percent of patients with high-grade IV to V spleen injuries, but they were otherwise similar (Table 1).

FNOM rates of the two NOM groups (NO-AE vs. AE) were stratified by grade (Table 2). There was no significant difference in FNOM for grade I to III injuries, but a significant decrease was noted in FNOM with the addition of AE for high-grade (IV–V) injuries. The two groups of grade IV to V patients for NO-AE and AE were well matched with no significant differences in the following factors: male (62% vs. 69%), age (37 vs. 37), ISS (26 vs. 27), Abbreviated Injury Scale (3 vs. 3), discharged home (71% vs. 67%), and admission SBP (119 vs. 115). Among the 94 total high-grade injuries, FNOM significantly decreased from 33% to 7% (p =0.009) with the addition of AE.

There were 104 AE patients. The technique was PMSAE in 65 patients (62%), SDSAE in 1 patient (1%), and both PMSAE and SDSAE in 38 patients (37%). The indica-

TABLE 2. Comparison of Failure of NOM Rates for NO-AE vs. AE Based on Grade of Splenic Injury

Grade	NOM Groups							
	NO	O-AE						
	Patients (No.)	Failed NOM (%)	Patients (No.)	Failed NOM (%)	p			
I	175	1	2	0	1.00			
II	124	2	17	0	0.32			
III	63	6	24	0	0.56			
IV	25	23	38	3	0.04			
V	8	63	23	9	0.03			

^{*} p < 0.05, OM vs. NO-AE.

p < 0.05, OM vs. AE.

p < 0.05, NO-AE vs. AE.

NS, no significant differences between the three groups.

tion for AE was CB (77 patients [74%]), high-grade IV to V injuries (15 patients [14%]), and decreasing hemoglobin levels (11 patients [11%]). One additional patient (1%) underwent AE before heparinization for a pulmonary embolism. Of the 77 AE for CB, 43 (56%) also had high-grade IV to V injuries and therefore had dual indications for AE. All 11 AE for decreasing hemoglobin levels were low-grade I to III injuries. All 11 remained hemodynamically stable and underwent AE at various intervals during the first 48 hours of NOM observation based on rate of hemoglobin level decrease during serial evaluations. The mean starting hemoglobin level was 11.6 and mean ending hemoglobin before AE was 6.9. The range of hemoglobin was 12 to 6.4. All 11 patients had successful salvage (100%) with improvement of hemoglobin levels after AE. A small number of the 435 patients in the NO-AE group met criteria for AE but were not embolized (per attending surgeon on call decision): CB (7 patients [2%]) and high-grade IV to V injuries (31 patients [8%]). Three of the seven with NO-AE and CB also had high-grade IV to V injuries (dual indications), and all three (100%) FNOM. A significantly lower FNOM was found after AE versus NO-AE for CB-positive patients (5% vs. 71%, p = 0.002).

The average hospital day that FNOM occurred for all 23 failures was 2 (range, 1–5). Eleven (58%) of the 19 FNOM NO-AE group had high-grade BST (grades IV–V) and 5 (26%) were hemodynamically unstable (NIH/WTA grades III–V) on admission (Table 3). Five (26%) in this group who

were NO-AE, despite clearly having a CB and moderate- to high-grade BST (grade III in 2, grade IV in 3), all failed within 48 hours of NOM observation. Two of the FNOM AE group (50%) had grade V BST and were hemodynamically unstable on admission (NIH/WTA grades III-V). One of the remaining two FNOM AE patients had a nonbleeding grade IV injury for which laparotomy was performed after 2 days due to increasing abdominal pain but required splenectomy for an iatrogenic laceration. The fourth patient who had FNOM following AE was transferred from an outside institution with initial SBP 118 mm Hg. The patient was appropriately sent for AE based on CB on CT, however, soon thereafter became hemodynamically unstable and was emergently taken to the OR for splenectomy. Although the attending surgeon was not aware of the events at the outside facility, later retrospective review of the records identified multiple episodes of SBP < 90 mm Hg recorded before transfer. The patient received four units of blood transfusion before achieving a stable SBP for transfer (NIH/WTA grade IV).

Multiple logistic regression analysis showed that grade IV to V injuries and presence of CB were statistically significant (p < 0.05) independent risk factors for FNOM. Odds ratio calculations show that hemodynamically stable patients with a CB have a 22 times greater likelihood of FNOM if they are observed without AE (95% confidence interval: 10.8 < 21.7 < 43.6), and grade IV to V injuries have a 5 times

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NO-AE group 65 24	2006 2009 2007	M M	86	1 1				
65 24	2009 2007	M		1				
	2007			-	MCC	2	No	No
22			100	2	MVC	5	No	No
22		M	139	2	MVC	1	No	No
47	2000	M	132	2	MVC	2	No	No
27	2003	M	60	3	MVC	1	Yes	No
44	2004	M	151	3	MVC	2	Yes	No
24	2010	M	131	3	MCC	1	No	No
33	2001	F	80	3	MVC	1	No	No
42	2001	M	100	4	MVC	3	No	No
52	2003	M	141	4	SPORT	3	No	No
63	2003	M	121	4	MVC	4	No	No
43	2005	M	112	4	FALL	3	No	No
28	2007	M	125	4	PEDS	2	Yes	No
64	2009	F	142	4	MVC	1	Yes	No
46	2005	F	103	5	ATV	2	Yes	No
57	2007	M	115	. 5	PEDS	2	No	No
28	2007	F	70	5	MVC	2	No	No
34	2010	F	96	5	SPORT	1	No	No
44	2010	M	158	5	MCC	3	No	No
AE group								
22	2006	F	111	4	FALL	2	Yes	Yes
23	2007	M	118	. 5	MVC	2	Yes	Yes
53	2003	F	87	5	MVC	1	Yes	Yes
49	2007	F	76	5	MCV	1	Yes	Yes

MVC, motor vehicle crash; MCC, motorcycle crash; ATV, all-terrain vehicle; PEDS, pedestrian vs. automobile crash; HD, hospital day. Highlighted cells show inappropriate criteria for NOM.

greater likelihood of FNOM with observation and no AE (95% confidence interval: 1.6 < 4.7 < 13.8).

There were 40 deaths among the 539 NOM patients (7.4%), with no difference in mortality rates between the AE and NO-AE groups (Table 1). Only one death was directly attributable to the splenic injury (0.2% of all 539 NOM patients, 2.5% of all 40 deaths), a patient in the FNOM-AE group inappropriately sent to IR with hemodynamic instability and a grade V injury. All other deaths were due to complications of multisystem trauma.

No AE-related complications occurred in any of the study patients by the time of hospital discharge.

DISCUSSION

Recent reports of the potential for AE to further improve the success of NOM have resulted in its adoption at many centers.^{6–9} However, the actual benefit of this modality, and its indications, remains unclear. At our institution, protocols based on selective use of AE for high-risk groups were adopted and closely followed by all trauma surgeons for the past decade. Our compliance with the protocols is demonstrated by the steady proportion of patients who underwent OM (35%) versus NOM (65%) each year over the last decade (Fig. 1). Although this proportion corroborates that reported in the multi-institutional Eastern Association for the Surgery of Trauma (EAST) study published in 2000, our overall FNOM rate of 4.3% is substantially lower than that reported in the EAST study (11%); the FNOM rate for each injury grade is also significantly lower than EAST (Table 4).16 AE was rarely used during the study period of the EAST report. Our data indicate that the selective application of AE to high-risk BST patients to extend the application of NOM and the appropriate selection of patients for NOM were the major factors contributing to this improvement. Furthermore, our results were achieved with minimal spleen-related mortality (0.2%) and no AE-related complications during the period of hospitalization. One limitation of this study is the absence of any posthospital long-term follow-up.

Our protocol emphasized three main factors for success: (1) hemodynamically unstable patients belong in the OR; (2) selective use of AE for patients at high risk for failure (CB on initial CT, high-grade injuries [IV–V] on initial CT, and/or decreasing hemoglobin levels during NOM observation) rather than universally for all patients with BST; and (3)

TABLE 4. Comparison of UF FNOM Rates With EAST Study¹⁶

	E	AST	UF		
Grade	Patients (No.)	Failed NOM (%)	Patients (No.)	Failed NOM (%)	p
I	267	5	177	1	0.01
II	299	10	141	2	0.01
III	247	20	87	5	0.002
IV	194	33	63	11	0.002
V	78	75	31	26	0.02

delayed AE can be safely performed to salvage hemodynamically stable patients with decreasing hemoglobin levels.

The most important principle to follow in NOM of BST is that all hemodynamically unstable patients require immediate surgery for hemorrhage control. NOM should be contraindicated in these patients. This was best illustrated by the fact that in this retrospective evaluation, 5 of the 19 FNOM NO-AE and 3 of the 4 FNOM with AE patients were hemodynamically unstable by the NIH/WTA criteria^{14,15} during their initial resuscitation and should never have been nonoperatively managed. This predictably resulted in FNOM of all eight patients (and the only spleen-related death) within 48 hours, which should be considered one marker of poor patient selection. The most common reason documented for this ill-guided decision was that the patients were transient responders to volume resuscitation before initial CT, demonstrating the pitfall of relying on single blood pressure readings rather than the overall condition of the patient in making a decision for NOM. The higher ISS of the AE group (Table 1) may have been partially due to the inclusion of these inappropriately selected patients, but their more severe injuries emphasize the fine line that surgeons must walk in making this critical decision for NOM. AE should not be considered a valid treatment option for hemodynamic instability. Had more appropriate patient selection occurred, our overall FNOM rate could have been reduced to 1.8% (10/539).

Peitzman et al.¹⁷ documented the dangers of poor patient selection for NOM of BST in their report of 78 patients with FNOM in the EAST Multicenter study. Twenty-five percent of these patients had been unstable on admission, and 10 patients (12.6%) had spleen-related mortality. The mortality rate was 37% among the unstable patients and 3% among the stable patients. An undue delay in laparotomy was responsible for 60% of the deaths and three patients exsanguinated without laparotomy. Five (22%) of our 23 FNOM patients in this study failed within the first 24 hours of observation. Three of our eight unstable FNOM patients died, but only one was spleen-related. This 12.5% (1/8) spleenrelated mortality among our unstable NOM patients compares favorably to the 37% mortality rate among unstable NOM patients reported by Peitzman et al.¹⁷ Nonetheless such deaths should be considered preventable and emphasize the importance of adhering to strict selection criteria to optimize the safety of NOM for BST.

Another lesson was illustrated by our patient described above in Results who had been transfused four units of packed cells to maintain his blood pressure and then quickly failed NOM. A study of the role of blood transfusion in BST by Velmahos et al.¹⁸ identified, among other variables, that transfusion of more than one unit of blood was an independent risk factor for FNOM. In their study, patients with grade III to V BST that received more than one unit of blood transfusion during NOM had a 100% FNOM rate.

AE should be applied in a selective manner based on high-risk factors for FNOM (CB on initial CT, high-grade injuries [IV–V] on initial CT, and/or decreasing hemoglobin levels during NOM observation) rather than universally for

all patients with BST. This was first suggested in 1995 by Sclafani et al.9, one of the earliest studies of AE for BST. All 150 hemodynamically stable BST patients undergoing NOM in this study underwent angiography. The majority of patients underwent PMSAE with minimal use of SDSAE. The splenic salvage rate was 98.5%, one of the lowest FNOM rates ever reported and since then unmatched. Sixty patients had positive angiograms and underwent AE. Ninety of the 150 patients (60%) had negative angiograms, all of which may have been unnecessary since 85 of the 90 (94%) patients had low-grade (I–III) injuries, and the overall FNOM rate was 0% with simple observation. Similarly, we found in our study that patients with grade I to III injuries did not have a significant decrease in the FNOM rate after AE while those with grades IV to V did (33% to 7%, p = 0.009). On the basis of these data, we recommend routine AE of all grade IV to V injuries, regardless of any other finding, using selective embolization for grades I to III only if they manifest CB and/or decreasing hemoglobin levels during NOM observation.

Haan et al.¹⁹ reported in 2005 an aggressive angiography and AE protocol that resulted in the successful NOM of more than 80% of high-grade (IV–V) injuries. The authors compared their approach with that of Sclafani et al. and concluded that although the results were similar, only minimal utility was found in the use of angiography for low-grade (I–II) injuries.^{9,19} Furthermore, their protocol was modified to a more selective use in those with grade III to V and/or vascular injuries as a result of these findings. Similarly, we found in this study that 94 patients in the NOM group had grade IV to V injuries, of which 15 were FNOM (16%), for an 84% success rate. We agree with the approach proposed by Scalea et al. with the additional recommendation, supported by the data reported herein, that AE be performed in all patients with grade IV to V injuries.

Previous studies have also found that the presence of CB on CT is a poor prognostic indicator resulting in increased FNOM rates.^{4,20} Fabian et al. reported that the presence of CB on initial CT predicted 24 times the likelihood of FNOM, similar to our finding of a 22-fold increased FNOM in this setting.⁴ The combination of CB and/or grade IV to V injuries is felt by some authors to be a contraindication to NOM.4,5 Thompson et al.⁵ reported a high rate of FNOM of BST in patients with both high injury grade and CB greater than 1 cm in size. In fact our results show this as well in that the three patients with this combination undergoing NOM without AE all failed. However, in the 44 NOM patients with this combination who had AE, only 4 failed (100% vs. 9%, p = 0.02). Our data support that the addition of AE to NOM has a distinct benefit in allowing the safe extension of NOM to high-risk groups that previously were not deemed acceptable for this management option.21

Our data further show that AE can be performed safely in a delayed manner in NOM of patients who remain hemodynamically stable but have decreasing hemoglobin levels. We had 15 such patients who had no CB and did not undergo initial AE. All 15 (100%) were salvaged with the hemoglobin stabilizing immediately after AE. Interestingly, 5 of these 15 were high-grade (IV–V) injuries and would have benefited

from AE initially based on grade alone, further justifying the application of AE to all high-grade injuries.

Finally, the type of AE (PMSAE vs. SDSAE) may provide some insight into why both the FNOM and complication rates have been decreased. There is a growing body of evidence that PMSAE may result in less complications and a higher success rate of NOM than SDSAE.9,22 In PMSAE, the main splenic artery is embolized with open coils. This results in decreased blood flow and lower splenic blood pressure, which facilitates clot formation and hemostasis, while still allowing some direct and collateral flow to maintain splenic viability. Human studies of PMSAE have demonstrated a decrease in splenic blood pressure of 47% and 58%.²³ SDSAE produces areas that have no collateral blood supply and may undergo necrosis and infection.²⁴ Sixty-two percent of the patients in this study underwent PMSAE, and another 37% had both PMSAE and SDSAE. The evolution of AE techniques is an important factor in achieving the high success rates and low complication rates of NOM of BST.

CONCLUSIONS

The application of strictly defined criteria for the addition of AE to NOM of BST was found to be safe and effective, resulting in one of the lowest reported FNOM (4.3%) and spleen-related mortality rates (0.2%). This study indicates that hemodynamically stable BST can safely undergo NOM and that the selective application of AE to the high-risk groups defined herein will extend the indications for, and success of, NOM for BST.

AUTHORSHIP

I.S.B., D.S., J.J.T., and A.J.K. designed this study. I.S.B. conducted the literature search. I.S.B., D.S., and J.P. collected data, which I.S.B., E.R.F., D.S., D.C., J.P., and A.J.K. analyzed. I.S.B., E.R.F., D.S., D.C., J.J.T., and A.J.K. interpreted the data. D.C. performed statistical analyses. I.S.B., E.R.F., D.C., J.J.T., and A.J.K. wrote the manuscript; I.S.B. and A.J.K. prepared figures and tables. I.S.B., E.R.F., J.J.T., and A.J.K. edited the final paper.

DISCLOSURE

The authors declare no conflicts of interest.

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