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Contrast-enhanced ultrasonography to diagnose complicated acute cholecystitis

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Abstract Gangrenous cholecystitis and perforation are severe complications of acute cholecystitis, which have a challenging preoperative diagnosis. Early identification allows better surgical management. Contrast-enhanced computed tomography (ceCT) is the current diagnostic gold standard. Contrast-enhanced ultrasonography (CEUS) is a promising tool for the diagnosis of gallbladder perforation, but data from the literature concerning efficacy are sparse. The aim of the study was to evaluate CEUS findings in pathologically proven complicated cholecystitis (gangrenous, perforated gallbladder, pericholecystic abscess). A total of 8 patients submitted to preoperative CEUS, and with subsequent proven acute complicated cholecystitis at surgical inspection and pathological analysis, were retrospectively identified. The final diagnosis was gangrenous/phlegmonous cholecystitis (n. 2), phlegmonous/ulcerative changes plus pericholecystic abscess (n. 2), perforated plus pericholecystic abscess (n. 3), or perforated plus pericholecystic biliary collection (n. 1). Conventional US findings revealed irregularly thickened gallbladder walls in all 8 patients, with vaguely defined walls in 7 patients, four of whom also had striated wall thickening. CEUS revealed irregular enhancing gallbladder walls in all patients. A distinct wall defect was seen in six patients, confirmed as gangrenous/phlegmonous cholecystitis at pathology in all six, and in four as perforation at macroscopic surgical inspection. CEUS is a non-invasive easily repeatable technique that can be performed at the bedside, and is able to accurately diagnose complicated/perforated cholecystitis. Despite the limited sample size in the present case series, CEUS appears as a promising tool for the management of patients with the clinical possibility of having an acute complicated cholecystitis.

Keywords Contrast-enhanced ultrasonography · CEUS · Complicated cholecystitis · Gallbladder perforation · Gangrenous cholecystitis · Pericholecystic abscess

Introduction

Gangrenous cholecystitis is an advanced form of acute cholecystitis, accounting, in some series, for up to 30 % of acute cases [1]. This form may progress to severe necrosis of the gallbladder wall with consequent perforation, leading to a high and increased morbidity and mortality in comparison with less complicated cholecystitis. The mortality rate for acutely perforated cholecystitis is reported to be up to 43 % [2]. An open surgical intervention rather than a laparoscopic approach is often required, and percutaneous drainage must be carried out in patients unfit for surgery [1, 3]. Imaging or clinical/laboratory findings that allow a rapid and precise preoperative diagnosis of gangrenous perforated cholecystitis is highly desiderable to aid the surgeon in prioritizing patients for operation.

Unfortunately, the signs and symptoms are often elusive in complicated cases, and differentiation from those not complicated is challenging, due to the lack of definite clinical or laboratory data. Thus, imaging still maintains a pivotal role.

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B-mode ultrasound (US) is the first-line imaging study in patients with suspected gallbladder disease, as this modality is sensitive and specific for acute cholecystitis, but it is of limited value in complicated cases. Abdominal contrast-enhanced computed tomography (ceCT) scan is utilized in patients with an acute abdomen when sonography is inconclusive, and it is regarded as the mainstay modality for complicated cholecystitis. Its accuracy in the detection of acute gangrenous cholecystitis has been reported to range from 80 to 92 % for the detection of wall perfusion defects [4, 5].

Contrast-enhanced ultrasound (CEUS), by means of a purely intravascular contrast agent, allows a real-time safe examination of the gallbladder walls' perfusion, directly at the bedside, even in severely ill patients or in cases with renal insufficiency, conditions which are frequently found in patients with sepsis of biliary origin, and which often make a ceCT contraindicated [6].

According to the 2011 EFSUMB guidelines and clinical practice recommendations about the use of contrast-enhanced ultrasound in non-hepatic applications, the detection or exclusion of abscess formation in the liver parenchyma surrounding the gallbladder can be performed with CEUS [6]. A wall perforation is suggested by an interruption of the perfused gallbladder wall, appearing as an enhancement defect, but data from the literature about the accuracy of this study are sparse, and recoμendations are mainly based on expert opinions.

A recent article by Tang et al. [7] describes an Asian population with six cases of complicated cholecystitis in whom contrast-enhanced ultrasound clearly visualized defects in the gallbladder walls or revealed pericholecystic abscesses.

The purpose of this study is to describe our single-institution experience about the CEUS findings in a series of patients with pathologically proven acute complicated cholecystitis (gangrenous or perforated gallbladder with/without pericholecystic abscess), to further validate diagnostic criteria proposed by the recommendations and confirmed by the Eastern series, as well as in a series of Western patients from our own center [7].

Methods

Patients

The possibility of the use of CEUS in the setting of acute cholecystitis has been adopted in our center for a long time, and this experience has also been incorporated in the EFSUMB guidelines as expert opinion [6]. Patients who underwent CEUS after a first B-mode ultrasound (US) examination in the clinical appearance of an acutely complicated cholecystitis were retrospectively identified from the electronic patients' folders database at our Institution, from 2008 to May 2013. Inclusion criteria were as follows: (a) having received a CEUS examination of the gallbladder (b) CEUS had to be performed after a B-mode US for a clinical appearance of a moderate to severe acute cholecystitis, according to the Tokyo diagnostic criteria [8] (c) and availability of the most relevant images and clips digitally recorded and stored. All patients signed a consent form before being injected with contrast for CEUS. This process yielded the identification of 13 patients with moderate to severe acute cholecystitis who had been studied with CEUS. Patients diagnosed with malignancy at histopathological results or without a pathological specimen as a diagnostic reference were excluded.

In order to have the most solid reference standard, namely histology, we retrospectively searched for patients who underwent CEUS for complicated acute cholecystitis with gangrenous or perforated gallbladder, either with or without a pericholecystic abscess, and who were subsequently operated upon. The surgical findings and pathological specimen of the gallbladder served as the gold standard. Gangrenous changes as a reference standard were defined on the basis of transmural acute inflammation with necrosis/gangrene of the gallbladder walls, while perforation was diagnosed on the basis of macroscopic discontinuity of the gallbladder walls. This search produced eight patients. The other five patients were excluded since four patients did not undergo surgery, while one patient underwent surgery, and was diagnosed with uncomplicated acute cholecystitis.

The study protocol was in keeping with the latest Helsinki declaration and was communicated to the Institutional Review Board of the hospital. Informed consent was obtained whenever patients were still reachable, otherwise it was accepted to have it waived, given the retrospective approach of the study.

US and CEUS examination

Conventional sonography of the gallbladder was performed with a curved array 3.5–5 MHz transducer (Esaote, Genova, Italy) in B-mode. CEUS was performed using SonoVue as contrast agent (Bracco SpA, Milan, Italy), a suspension of hexafluoride gas forming microbubbles with a diameter from 0.5 up to 1.5 micrometer. The bubbles are expelled through the pulmonary capillary bed, and act as a pure intravascular contrast agent. A total of 2.4 ml of contrast agent was administered to all patients through a peripheral vein, followed by a flush of 5–10 ml of sterile saline solution as per standard procedure. The enhancement pattern of the gallbladder wall was divided into an arterial phase (about 0–30 s after the injection) and a venous phase

(from about 31 s onwards from injection) as the gallbladder is only supplied by the cystic artery without any portal contribution. The assessment of the gallbladder and of the surrounding liver parenchyma was carried out for at least 3 min after contrast injection. The most relevant images and clips had been digitally recorded and stored. CEUS was performed in all cases with a curved array low-frequency (3.5–5 MHz) probe. CEUS with linear high-frequency (up to 10 MHz) probe was further performed on an individual basis to reach a better definition of tiny irregularities when the gallbladder walls were close (2–4 cm deep) to the abdominal wall.

The examiners were physicians experienced in ultrasonography and CEUS, who were aware of the clinical findings.

Definition of imaging findings

The following findings are routinely evaluated on conventional US in this setting: presence of gallstones, intraluminal material (irregular intraluminal sludge/debris), hydrops (transverse distension of gallbladder >4 cm), increased wall thickness (>4 mm), irregular wall thickness, vaguely discernible or blurred walls, striated wall thickening, suspected abscess (inhomogeneous hypo-hyperechoic mass surrounding the gallbladder wall), and pericholecystic fluid.

The findings evaluated on CEUS, in keeping with the most recent recommendations were interruption/defect of the gallbladder wall confirmed by the focal absence of enhancement or detection of abscess in the surrounding liver parenchyma [6]. The latter was reported as a pericholecystic mass, showing the typical feature of non-enhancing areas possibly surrounded by an area of peripheral enhancement in the arterial phase expressing inflammatory hyperemia in the absence of clear marked wash out of the contrast medium in the venous phase [9]. As reported by previous studies on CT scan, which show irregular enhancing gallbladder walls as a specific sign of pathologically proven gangrenous changes, we also evaluated if an irregular contrast enhancement of gallbladder walls was noticed on CEUS, described as areas of persistent lack of enhancement within the mucosal layer of the gallbladder, considered as a potential sign of necrosis of the wall, even if a clear transmural enhancement defect was not seen [5, 10]. Contrast-enhancement appearance of intraluminal echogenic material observed on B-mode US, likely gallbladder sludge or debris, was also recorded.

To the main interest of the study, namely to verify whether there was an interruption in the perfused gallbladder walls, both vascular phases were of interest, since a persistent lack of perfusion had to be demonstrated in both the arterial and venous phases. Thus, the patients were studied for at least 3 min, to assess the entire area, which comprises both the arterial and venous phases.

The main laboratory findings were collected from the patients records within 24 h from CEUS.

Five out of the eight patients also underwent a ceCT scan within 24 h from CEUS. In keeping with the findings considered for CEUS, the following findings from ceCT scan reports were then collected and compared with that of CEUS: irregular or absent walls (irregular contrast enhancement) and pericholecystic abscess (encapsulated fluid collection adjacent to gallbladder).

Results

A CEUS study was performed only in patients with the clinical/laboratory appearance of complicated cases of acute cholecystitis, and the surgical specimen with pathological diagnosis was chosen as the gold standard reference. The initial study group consisted of 13 patients who underwent a CEUS study for the appearance of moderate to severe acute cholecystitis. The CEUS study suggested complicated acute cholecystitis in 10 out of those 13 patients. All 10 cases with a positive CEUS study were recommended to have surgery. However, based on clinical decisions, only 8 out of 10 patients with a positive CEUS study for complicated cholecystitis finally underwent surgery, all were confirmed to be complicated acute cases. Of two remaining patients out of 10 cases with a positive CEUS study, one was managed conservatively with antibiotic and supportive therapy because of comorbidities. He recovered and was discharged after 10 days, although we were subsequently informed he was readmitted for abdominal pain 1 month later in another hospital, but we have no further information. This might have been an uncomplicated case, to be categorized as a CEUS false positive study, but a solid reference standard lacks. The other patient underwent CT-guided percutaneous drainage, (because of severe comorbidities that prevented laparotomy), after a contrast-enhanced CT scan that also suggested a complicated gangrenous cholecystitis, so its likelihood of being a false positive case appears very low. Conversely, three patients were deemed not to suffer from complicated cholecystitis at the CEUS study. One of them was operated within 2 weeks, and the pathology confirmed an acute non-complicated cholecystitis (true negative case), while the remaining two were managed conservatively without surgery, in keeping with the CEUS study interpretation (they both recovered and were discharged on 16th and 8th day after the CEUS studies, respectively). However, again in these cases, a solid reference standard

(like surgical inspection and pathology) was lacking, so that false negative cases of complicated cases resolved with medical therapy cannot be ruled out. Therefore, the final analysis was focused on the 8 patients (5 men, 3 women, median age 68 years, range 48–89) who received surgery with the the clinical appearance of acute complicated cholecystitis, a diagnosis confirmed by the reference standard in all cases. The Laboratory data in these 8 patients showed a median white blood cell count and C-reactive protein of 12.620/mm^3 (range 4990–23,450) and 30 mg/dl (range 12–34, normal value <0.80 mg/dl), respectively.

Surgical and Pathological findings

The main surgical, pathological, and imaging findings are reported in Table 1. Surgical macroscopic and pathological diagnosis of acute complicated cholecystitis consisted of gangrenous/phlegmonous changes (n. 2), phlegmonous/ulcerative changes plus pericholecystic abscess (n. 2), perforated plus pericholecystic abscess (n. 3), or perforated plus pericholecystic biliary collection (n. 1). All eight cases taken to surgery for the diagnosis of acute complicated cholecystitis were all operated with open surgery (no laparoscopic attempt). The median time between the CEUS study and surgical intervention was 4 days (ranging from a minimum of 1 to a maximum of 13 days). No cases of malignancy were detected at pathological diagnosis.

B-mode US findings

The conventional US findings of our series of pathologically proven complicated cholecystitis revealed irregularly thickened gallbladder walls in all 8 patients, with vaguely defined walls in 7 patients, four of whom also had striated wall thickening. All patients showed gallbladder wall thickness >4 mm at least in some parts. Gallbladder hydrops was seen in 7 patients, while pericholecystic fluid effusion was only found in 3 patients. Gallbladder stones and intraluminal membranes/debris were both present in 7 patients. A pericholecystic mass abscess was suspected in 3 patients.

CEUS study findings

During the arterial phase, the gallbladder walls became irregularly enhanced in all 8 patients, in particular the inner mucosal layer was involved in all cases, with heterogeneous areas of lack of contrast enhancement. A clear interruption of both inner-outer mucosal layers with a total transmural defect was observed in 6 patients, varying in five patients between a length of 5 mm up to 20 mm (Figs. 1, 2, 3, 4, 5), while in the sixth patient, B-mode US showed thinning of the gallbladder wall at the body/fundus associated with a large pericholecystic fluid collection. The CEUS study showed no enhancement at all along the thinned tract of the gallbladder wall, suggestive of covered perforation (Fig. 6). A clear perforation was confirmed by macroscopic exploration at surgery in 4 of those 6 patients (Figs. 2, 3, 4, 6) (time from CEUS study to surgery: 7, 1, 10, and 2 days), including the suspected covered perforation (Fig. 6). In the two remaining patients with an enhancement defect of the gallbladder wall at the CEUS study (Figs. 1, 5), intra-operative findings could not confirm macroscopic perforation (time from the CEUS study to surgery: 1 and 5 days), but these two, as well as the other four, were diagnosed as gangrenous/phlegmonous cholecystitis at pathology.

The two remaining patients, who did show only irregular contrast enhancement of gallbladder walls at the CEUS study, without a clear transmural defect, were confirmed as being ulcerative/phlegmonous cholecystitis, at surgery (Figs. 7, 8).

The CEUS study showed a pericholecystic, non-enhancing mass in 4 out of 8 cases. The two biggest masses measured 6 and 4 cm in diameter and appeared heterogeneous on the CEUS study, with intralesional septae (Figs. 4, 7) and were confirmed as abscesses on pathological specimens. Of the two remaining suspected pericholecystic masses, one (Fig. 5) did not show the typical CEUS study features of abscess (s.a. rim-like enhancement with septae), being described as a defect in the gallbladder walls with irregular adjacent mass (4 cm in diameter) raising the possibility of heteroplasia or a covered perforation, diagnosed as phlegmonous cholecystitis at pathology. The second case showed a small defect of contrast enhancement in the gallbladder walls, with tiny pseudodiverticular images of the fundus (Fig. 1), suggestive for initial abscess formation: on surgery it was diagnosed as gangrenous cholecystitis.

The intraluminal material observed at B-mode US in 7 patients, which could have been misdiagnosed as a solid mass by conventional US, appeared persistently as non-enhanced on the CEUS examination, suggesting biliary sludge then confirmed by pathology (Figs. 1, 2, 4, 7, 8).

One case with pathologically proven acute cholecystitis showed homogeneous contrast enhancement of the inner layer at the CEUS study, without any irregular areas of lack of contrast enhancement within mucosal layer (Fig. 8, Panel **b**).

The CEUS study with a linear high-frequency probe (up to 10 MHz) was performed in one case (Fig. 1), in which the fundus of the gallbladder was superficial and very close to the abdominal wall. In this case, the CEUS study markedly increased the ability to depict very small irregularities of the walls (less than 10 mm in size), with respect to CEUS with low-frequency (3.5–5 MHz) probe performed immediately before.

Table 1 Imaging findings at US, CEUS, and contrast-enhanced CT of complicated cholecystitis cases with surgical and pathology reports

Case n.	Patients number	Age (years)	Surgical findings	Pathology	Time admission-CEUS (days)	Time interval CEUS-Surgery	B-mode US Murphy sign	B-mode US Gallstones	B-mode US Hydrops	B-mode US Pericholecystic fluid
1	1	68	Empyema + perivisceritis	Gangrenous cholecystitis	4	1	Yes	Yes	Yes	No
2	2	89	Perforated gangrenous cholecystitis	Abscessualized cholecystitis	7	7	No	Yes	Yes	Yes
3	3	67	Perforated cholecystitis + pericholecystic abscess	Ulcerative gangrenous cholecystitis	2	1	Yes	Yes	Yes	Yes
4	4	86	Perforated gangrenous cholecystitis	Necrotizing acute cholecystitis + pericholecystic abscess	6	10	No	Yes	Yes	No
5	5	68	Acute cholecystitis	Phlegmonous cholecystitis	1	5	Nd	No	No	No
6	6	56	Perforated gangrenous cholecystitis + pericholecystic biliary collection	Phlegmonous acute cholecystitis + perivisceritis	5	2	Yes	Yes	Yes	Yes
7	7	48	Empyema + pericholecystic abscess	Ulcerative cholecystitis + perivisceritis	4	13	No	Yes	Yes	No
8	8	82	Empyema + pericholecystic abscess	Phlegmonous cholecystitis + perivisceritis	8	2	Yes	Yes	Yes	No

Case n.	B-mode US Intraluminal membranes	B-mode US Walls thickening (>4 mm)	B-mode US Irregular walls thickening	B-mode US Striated walls thickening	CEUS Irregular contrast-enhancing walls	CEUS Walls interruption	CEUS Pericholecystic abscess	ceCT Walls interruptio n	ceCT Pericholec ystic abscess
1	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes
2	Yes	Yes	Yes	No	Yes	Yes	No	Yes	No
3	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	No
4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	/	/
5	No	Yes	Yes	Yes	Yes	Yes	?[a]	Yes	No[b]
6	Yes	Yes	Yes	No	Yes[c]	Yes[c]	No	/	/
7	Yes	Yes	Yes	No	Yes	No	Yes	No	Yes
8	Yes	Yes	Yes	Yes	Yes	No	No	/	/

Nd not described in the report

[a] A pericholecystic mass was noted on CEUS, but without typical features of an abscess mass (s.a. rim-like enhancement), raising suspicion suggesting a perforation or heteroplasia

[b] ceCT describes gallbladder walls' ulceration plus a pericholecystic mass with pseudodiverticular image of the fundus

[c] On CEUS examination gallbladder walls are non-enhanced, hence likely necrotic and leaky

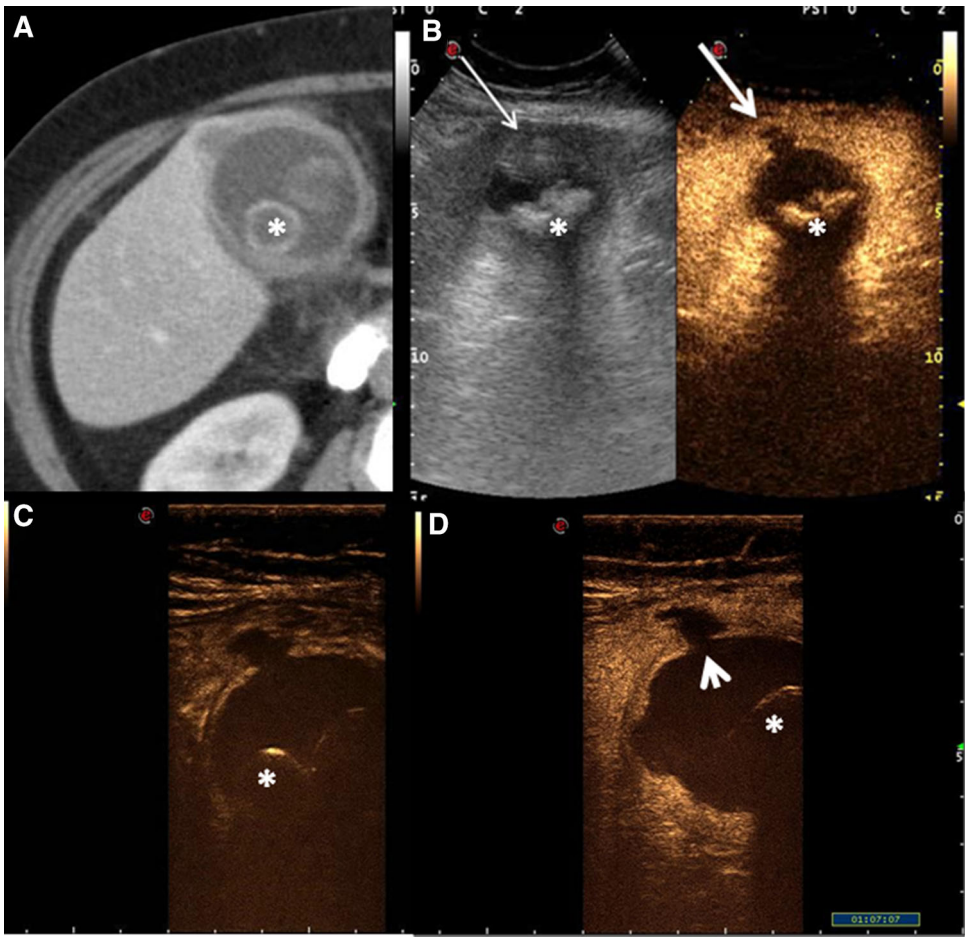

Fig. 1 Acute gangrenous cholecystitis in a 68-year-old woman. On panel **b** (dual imaging) CEUS shows a small defect in the fundus walls (*thick arrow*); B-mode US (Panel **b**, *left*) could not identify a discontinuity, but shows a vaguely indefinite wall's border with echogenic mass inside the lumen (*thin arrow*). On panel **c** (arterial phase) and **d** (venous phase) the same case, observed with a linear high-frequency probe (up to 10 MHz) that markedly increases the ability to depict the subcentimetric wall's discontinuity (*small arrow*), the tiny pseudodiverticular image (13 mm) suspected for being pericholecystic abscess and the absence of enhancement of intraluminal debris. On panel **a** the corresponding ceCT scan image shows the same features. *Asterisks* indicate gallstones

CEUS: ceCT findings

Five patients underwent a CT scan within 24 h from the CEUS study. A concordance beween the CT scan and the CEUS study was observed in all cases. In four patients, both techniques showed a transmural gallbladder wall defect, two of which were confirmed as perforated at surgical inspection. In one of those four patients, the CEUS study and the CT scan also showed a pericholecystic abscess, which was the only finding (again confirmed by both imaging methods) in the fifth patient.(Figs. 1, 2, 3, 5, 7; Table 1).

Discussion

The combination of clinical signs, laboratory tests, and conventional US usually leads the surgeon to the proper therapeutic choice in the instance of acute cholecystitis, but the decision about when a ceCT scan is additionally required for best management still remains challenging and debated.

Untreated acute cholecystitis may resolve within 7–10 days, but complications are common.

In patients with acutely complicated cholecystitis, early cholecystectomy or radiological drainage/cholecystostomy in patients unfit for surgery is indicated in order to avoid further local and systemic septic complications [1, 3, 11]. Conversely, in the instance of acute uncomplicated cholecystitis, a wait-and-see policy under medical therapy and a subsequent elective surgical approach is our practice. Although differentiating complicated from non-complicated cholecystitis remains pivotal in the management of these patients, achieving such diagnoses remains a challenging step and more tools would be welcome.

Acute complications of cholecystitis occur in up to 15 % of cases [11, 12]. Gangrenous cholecystitis is the most serious stage of gallbladder inflammation, following vascular compromise and gallbladder wall ischemia, eventually resulting in gallbladder necrosis and perforation. Older male patients (>50 years old) with a history of cardiovascular diseases or a leukocytosis greater than 17,000 cells/mm^3 have an increased risk of gallbladder gangrene and conversion of laparoscopic to open cholecystectomy [1,

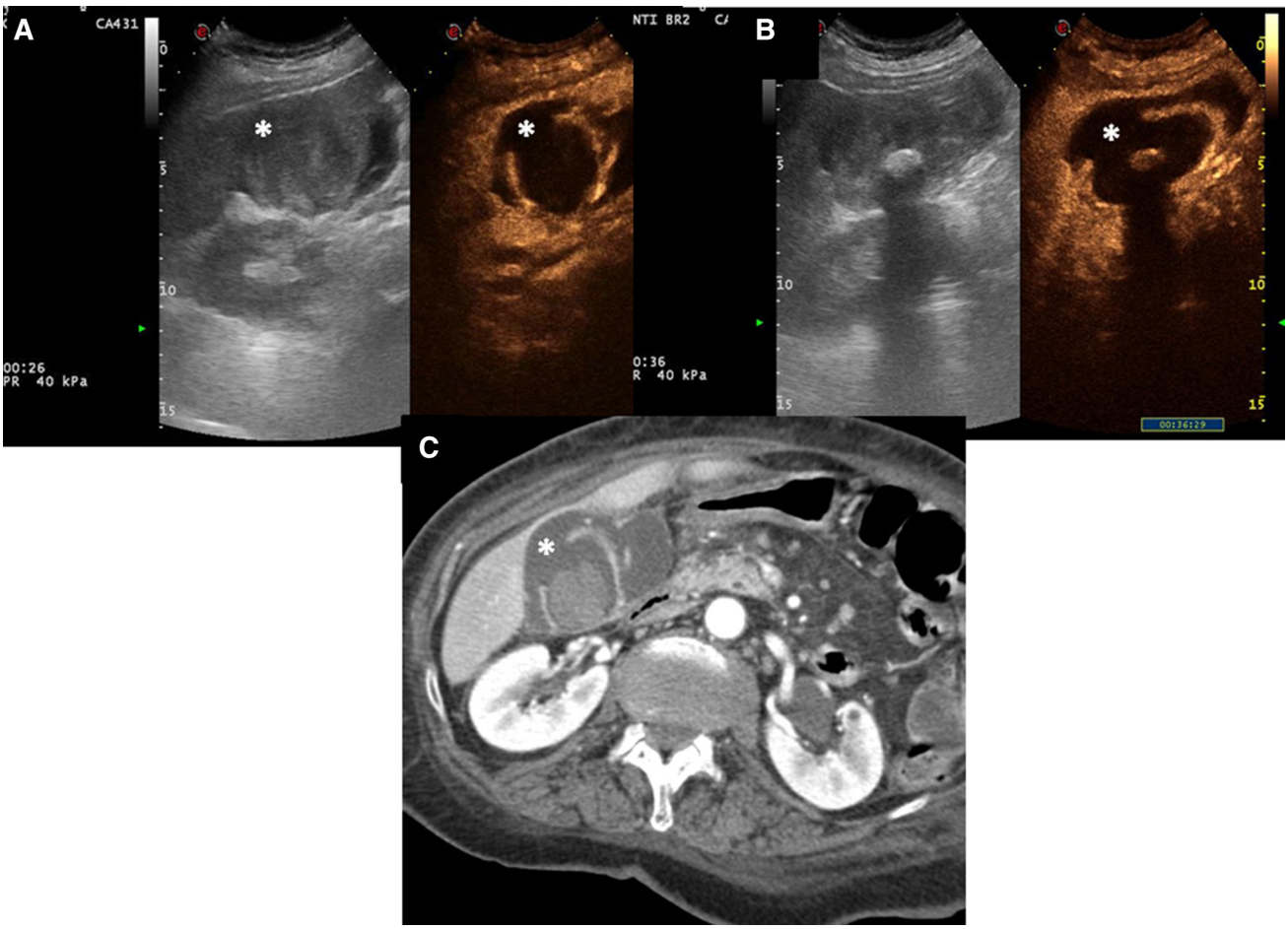

Fig. 2 A 89-year-old woman with gallbladder perforation. On dual imaging (panel **a**, **b**), real-time CEUS, on the right of each panel, shows a gross wall's defect in the gallbladder body (*asterisk*) in the arterial phase (26 s, panel **a**) and early venous phase (36 s, panel **b**), which is confirmed on ceCT image (panel **c**). Pericholecystic effusion, interruption in the gallbladder walls, and the absence of enhancement of the echogenic material within the gallbladder lumen (likely sludge) are clear at CEUS, while B-mode ultrasound (on the *left* of each panel) shows little pericholecystic fluid, vaguely defined gallbladder walls, and an apparently solid echogenic mass within the gallbladder lumen

13]. Borzellino et al. [14] recently found four factors to be independent predictive factors of severe acute cholecystitis in an emergency setting: fever, distension of the gallbladder, wall edema, and preoperative adverse events.

Beside clinical and laboratory findings, timely or delayed admission play a role in the progression toward complications of cholecystitis. Patients with mild complaints resulting in a longer duration of symptoms before admission are found to be associated with gangrenous cholecystitis [15].

Symptoms of complicated cases, except for obvious peritonitis or sepsis, are often non-specific. The absence of severe symptoms is not always a reassuring feature, as the Murphy's sign is occasionally reported to be absent, due to denervation of necrotized walls [16, 17]. An imaging modality that can suggest or even diagnose potential local complications, especially at the bedside and eventually lead to an urgent ceCT scan in doubtful cases, is eagerly desired. A CT scan is not pursued as a primary imaging technique for acute gallbladder disease in most countries, but is often obtained to evaluate for complications of acute cholecystitis, and it is frequently requested before surgery, especially in patients with a wider differential diagnosis or confusing signs/symptoms, to exclude other abdominal causes. Complications of acute cholecystitis have characteristic CT scan findings, including necrosis, perforation, abscesses, intraluminal hemorrhage, and wall emphysema. Defect in the gallbladder mucosa or sloughed intraluminal membranes suggests gangrene as well as focal transmural defect in the walls [4, 5].

The CT scan has some limitations, despite its important role in the detection of complications. The patient has to be referred to the radiology department; contrast injection is potentially contraindicated in patients with impaired renal

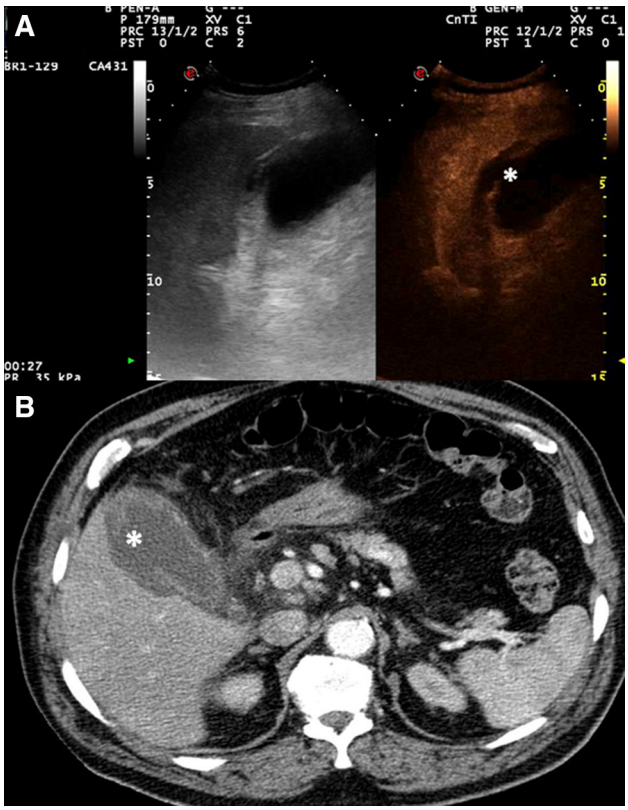

Fig. 3 A 67-year-old man with gallbladder perforation complicating acute cholecystitis. On panel **a** CEUS confirms the defect in the gallbladder wall (*asterisk*), while on B-mode US gallbladder walls are vaguely defined and irregularly striated and thickened (image on the *left* in the real-time dual imaging with CEUS). CEUS suggests a walled-off perforation, confirmed by contrast-enhanced CT scan and surgery

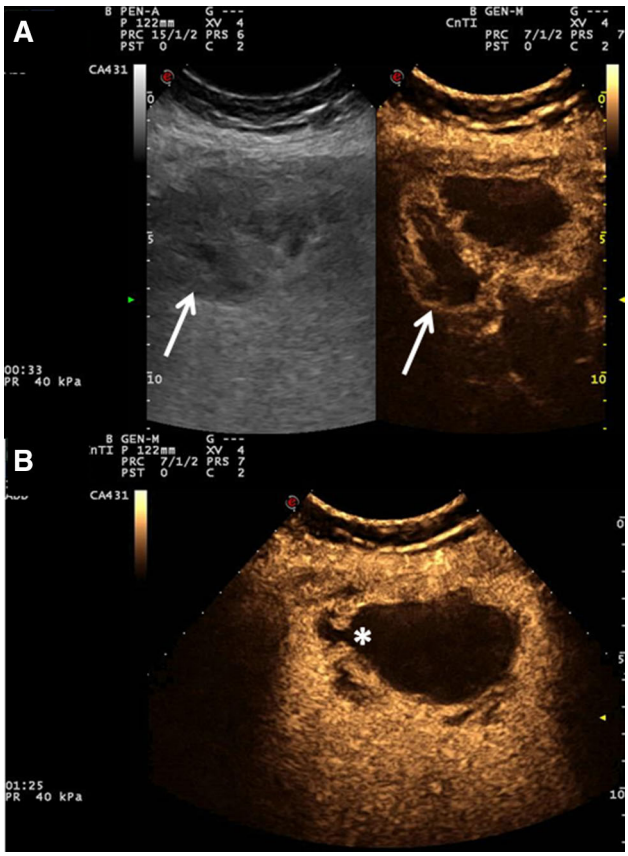

Fig. 4 A 86-year-old woman with perforated gangrenous cholecystitis complicated by a pericholecystic abscess. On B-mode US examination, a pericholecystic mass is hardly visible (*left side* of panel **a**, *arrow*), while bedside CEUS (panel **b** and *right side* of panel **a**) depicts the irregular arterial enhancement of the gallbladder walls along with a pericholecystic mass showing rim-like enhancement with a non-enhancing central area, consistent with abscess (*white arrow* on panel **a**). Note a tiny focal transmural wall defect (*asterisk*) of the gallbladder body on panel **b** and no enhancement of the intraluminal debris

function or hyperthyroidism; because of the administration of iodine content, it uses ionizing radiations and carries risk of some adverse allergic reactions.

B-mode US is the first-line imaging modality in patients with suspected gallbladder disease as this modality is sensitive and specific for acute cholecystitis, but in complicated cases it is of limited value. In fact it suggests potential complications on the basis of indirect signs such as free pericholecystic fluid, marked irregularity of the luminal surface, or heterogeneous walls with projections into the lumen [18–20], being the "hole sign" (a defect in the gallbladder wall) the only specific sign, but identified with variable and usually low sensitivity [21, 22], as confirmed in our cases.

In this setting, an ultrasound contrast agent, made of a purely blood pool tracer, which allows continuous, real-time examination of blood flow to organs, overcoming the limits of color-Doppler examination, is a very promising tool. The CEUS study is time-saving, allowing an immediate and safe bedside examination. CEUS does not require any laboratory investigation prior to contrast injection; it is associated with a rate of adverse events much lower than iodinated contrast agents and can be performed by clinicians immediately at the bedside and not only by radiologists, as in our case [6, 23]. Additionally, it might be particularly useful even when a CT scan is readily available, for those patients who have contraindications to contrast-enhanced CT scan because of renal failure or allergy to iodinated contrast media.

Although the CEUS study has been used to evaluate acute and chronic gallbladder disease, data about its usefulness in the detection of inflammatory complications of acute cholecystitis are sparse [10, 24–30]. Few reports focus on the diagnostic aid of a CEUS study in the detection of complicated acute cholecystitis such as gangrenous changes or perforation. Tang et al. [7] have recently described an Asian population with six cases of complicated cholecystitis in whom the CEUS study clearly

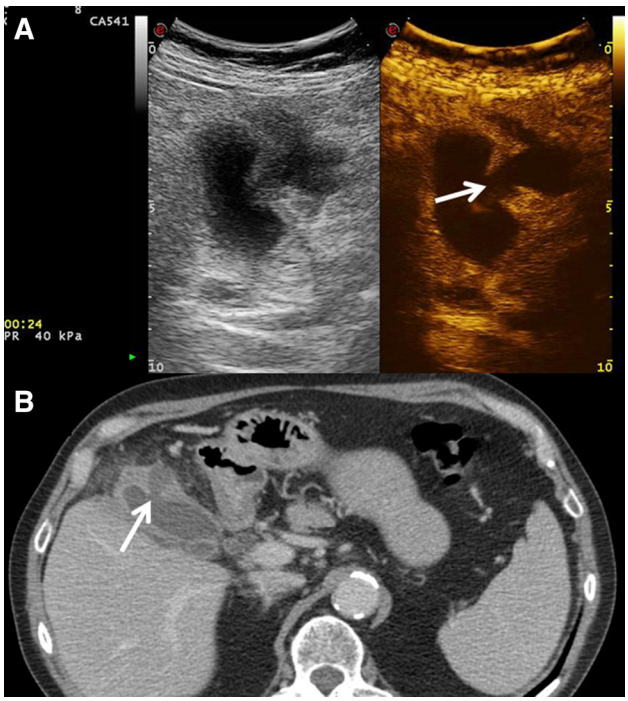

Fig. 5 CEUS of a 68 year old man shows a transmural defect in the body of the gallbladder (*white arrow* on panel **a**), clearly depicted at subsequent contrast enhanced CT scan (panel **b**), which also confirmed the pseudodiverticular mass around the fundus, which did not displayed typical features of abscess nor at CEUS nor at CT scan. The patient was diagnosed as phlegmonous cholecystitis at pathology (at surgical inspection a resection of gallbladder along with gallbladder bed was performed thus a covered perforation could not be macroscopically confirmed)

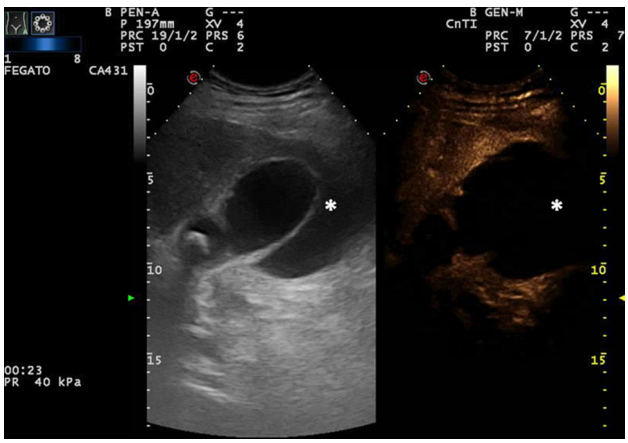

Fig. 6 A 56-year-old man with perforated cholecystitis complicated by pericholecystic biliary collection. On dual imaging, the B-mode US (*left frame*) shows cholecystic walls with progressive thinning toward the fundus (*asterisk*), without any clear interruption. On CEUS examination (*right*), gallbladder walls are totally non-enhanced, hence likely necrotic and leaky

visualized defects in the gallbladder walls or pericholecystic abscesses.

Our series reinforce these data and showed that irregularly contrast-enhanced gallbladder walls were consistent with a pathological diagnosis of gangrenous cholecystitis. In previous reports, CEUS using SonoVue (Bracco SpA, Milan, Italy) [31] or Sonazoid (Daiichi Sankyo, Tokyo, Japan), as contrast agents [32] shows a clear perfusion defect of the gallbladder wall in all cases of perforated cholecystitis.

Our data support the conclusions of previous studies about CT scan in gangrenous cholecystitis, in which an irregular gallbladder wall enhancement on ceCT scan was reported to be a specific sign of gangrenous changes, although it requires meticulous searching [4, 5]. An irregular contrast enhancement of gallbladder walls showing tiny focal defects within the inner mucosal layer was observed in all cases of gangrenous cholecystitis of our series, irrespective of the additional presence of perforation. The injection of ultrasound contrast media helped in clearly depicting the gallbladder wall borders, which instead on B-mode were irregularly and barely visible in the majority of cases. Our data are consistent with the results of ceCT studies that show that the accuracy of CT scan to discriminate between gangrenous and non-gangrenous cholecystitis is improved when patients are studied after intravenous administration of contrast agents [5]. Our experience also suggests that a CEUS study might be accurate in the depiction of a very small irregularity in the enhancement of gallbladder walls thanks to the real-time modality and high spatial resolution (especially when high-frequency transducers can be employed). In our personal experience, a CEUS study with an high-frequency probe can better depict very small (few millimiters in size) inhomogeneous areas in very superficial organs (fundus of the gallbladder, liver surface, etc.), when the region of interest is no more than 2–4 cm deep. Our case series also reinforces the importance of the CEUS scan in the characterization of intraluminal debris or sludge in the gallbladder, since CEUS is particularly useful in differentiation between gallbladder cancer and motionless biliary sludge. Motionless biliary sludge appears as echogenic solid material within the gallbladder, which is hard to differentiate from a solid mass by B-mode US alone. The CEUS study is extremely useful in ruling out the vascularization of intraluminal gallbladder masses; since biliary sludge lacks vessels, it is persistently non-enhanced on CEUS [26, 27, 29].

The presence of irregularly thickened or vaguely discernible walls at B-mode US is the most frequent sign associated with perfusion defects and pathologically proven complicated cholecystitis.

In the three cases of suspected uncomplicated acute cholecystitis at the CEUS study, one of which was pathologically proven to have been correctly diagnosed; the gallbladder walls showed early arterial homogeneous contrast enhancement of the inner mucosal layer, without

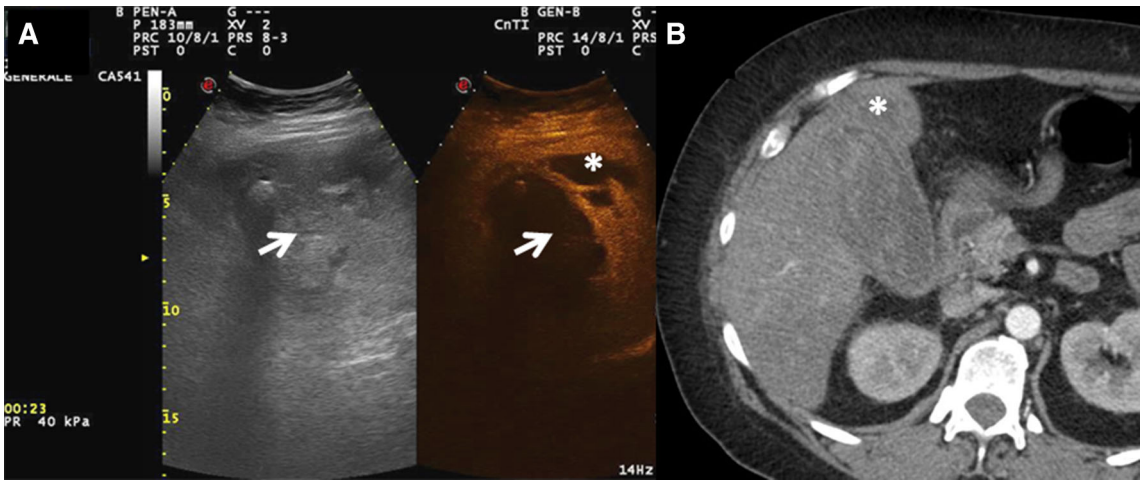

Fig. 7 A 48-year-old woman with ulcerative cholecystitis and pericholecystic abscess. Panel **a** dual imaging with B-mode US (*left side*) and CEUS (*right side*), during arterial phase. Note a pericholecystic abscess around the gallbladder fundus, appearing as a rim-like contrast-enhancing heterogeneous mass with non-enhanced central areas (*asterisk*) and enhanced septa. On panel **b**, corresponding contrast-enhanced CT scan confirms a pericholecystic abscess. The echogenic mass inside the gallbladder is persistently non-enhanced on CEUS (*arrows*), which was confirmed to be intraluminal sludge

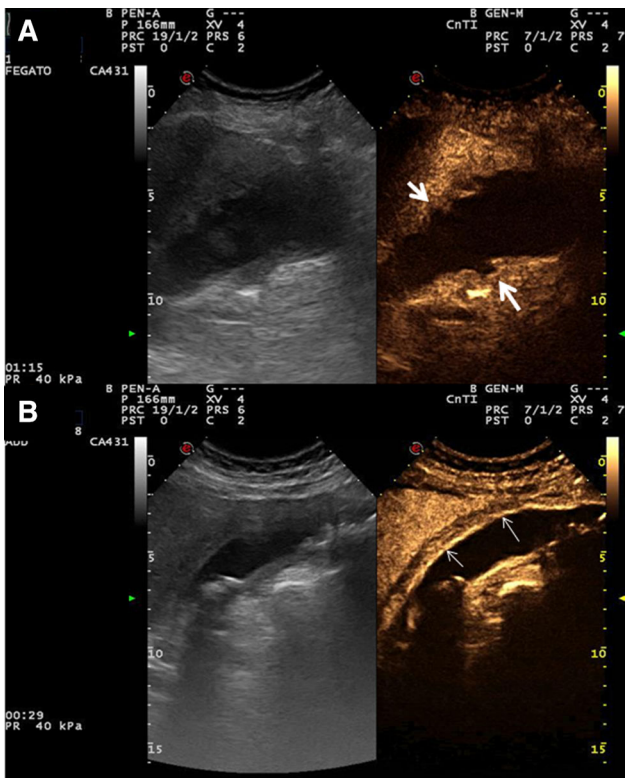

Fig. 8 Panel **a** an 82-year-old woman with pathologically confirmed phlegmonous cholecystitis complicated by perivisceritis. On B-mode US (*left*) vaguely discernible walls, with irregular thickening. On CEUS examination (*right*, here shown venous phase), the irregular enhancement of gallbladder walls became clearer, showing tiny persistently unenhanced (hence necrotic) areas within the inner layer of the walls (*thick arrows*). Panel **b** CEUS of non-complicated acute cholecystitis, showing homogeneous arterial enhancement of the inner mucosal layer (*thin arrow*)

perfusion defects within gallbladder mucosa, followed by progressive wash out in the late phase, compared with liver parenchyma. The two non-operated cases were then discharged without further complications after 16 and 8 days of hospitalization, respectively. These findings are in keeping with the data from literature showing early arterial contrast enhancement with a late phase wash out, while adjacent liver parenchyma is still enhanced as far as portal-early venous phases are ongoing in patients with acute uncomplicated cholecystitis [26, 30].

Despite the small sample size, we observed a full concordance between the CEUS study and CT scan either for the depiction of irregular or discontinuous contrast enhancement of gallbladder walls and the presence of pericholecystic abscesses. We emphasize that in our cases, some B-mode US findings previously reported to be suggestive of complicated cholecystitis, such as collection of pericholecystic fluid, were present in only three patients out of eight. Worrisome B-mode US findings raising the possibility of the walls' lack of integrity are an irregularly thickened wall (all cases), and vanishing or vaguely defined walls, in 7 out of 8 patients. Other B-mode aspects found in our complicated cases are intraluminal debris (7 out of 8) and hydrops (7 out of 8). However, these signs can also be found in uncomplicated cases. Only in three cases did B-mode US suggest a potential pericholecystic abscess.

The main limitations of our work are the limited sample size, the retrospective collection of cases, and the variable time between CEUS and surgery (median time 4 days, ranging between 1 and 13 days) that could have allowed for a series of confounding factors (s.a. antibiotic therapy) to influence the natural history of the disease.

The limited sample size is partially related to the need to obtain a gold reference standard such as histology. Indeed, in the absence of the histological gold standard, it is not possible to definitively classify CEUS patterns as false negative or false positive; thus, this initial report had to be limited to only patients who had undergone the reference standard. Clearly, following this choice, the included patients may represent only a percentage of the total number of patients with theoretical complicated cholecystitis treated during the period of cases collections. However, we point out that the CEUS study was performed only for moderate–severe cases and not in all cases of mild cholecystitis, which are the most frequent cases in real life in a Unit of Internal Medicine since data from studies report gangrenous cholecystitis in about 30 % of patients who undergo urgent surgery for acute cholecystitis [14, 15]. Since symptoms of gangrenous cholecystitis may be mild especially in older patients or with diabetes, it could be argued that many more cases with a CEUS pattern of complicated disease could have been collected if we would have performed a CEUS study in all the consecutive patients with acute cholecystitis referred to our hospital. Since it is not a standard approach yet, possibly only the most ill patients were imaged, and a prospective larger study is needed before a CEUS study should be included in the algorithm of complicated cholecystitis. Moreover, most patients with a suspicion of complicated cholecystitis have been likely directly admitted to the Emergency or Surgical Units rather than to our Unit of Internal Medicine, and CEUS is not yet routinely performed in those Units.

In conclusion, early identification of gangrenous or perforated cholecystitis is highly desirable in order to aid the surgeon to plan a timely treatment, but remains a challenging preoperative diagnosis, as no symptoms, laboratory findings, or B-mode sonographic findings are accurate enough. The findings of the present work in a Western population may stimulate physicians to consider the use of CEUS, if available at their facility, to help to quickly identify or to monitor cases of suspected complicated acute cholecystitis. This is of particular value in consideration of the extreme safety, low cost, and potential of bedside use of CEUS.

Conflict of interest Fabio Piscaglia has received speaker or advisory board fees or research support from Bracco, Esaote, Siemens, General Electric in the last three years. No other financial support was received from the industry from other authors.

Statement of human and animal rights The study protocol was in keeping with the latest Helsinki declaration and was communicated to the Institutional Review Board of the hospital.

Informed consent Informed consent was obtained whenever patients were still reachable, otherwise it was accepted to have it waived, given the retrospective approach of the study.

References

1. Merriam LT, Kanaan SA, Dawes LG et al (1999) Gangrenous cholecystitis: analysis of risk factors and experience with laparoscopic cholecystectomy. Surgery 126:680–685
2. Jain BK, Prasad D, Mohanty D, Garg PK, Diwaker P, Agarwal V (2012) Gallbladder perforation: a great masquerader. Am Surg 78:E30–E32
3. Navez B, Ungureanu F, Michiels M et al (2012) Surgical management of acute cholecystitis: results of a 2-year prospective multicenter survey in Belgium. Surg Endosc 26:2436–2445
4. Wu CH, Chen CC, Wang CJ et al (2011) Discrimination of gangrenous from uncomplicated acute cholecystitis: accuracy of CT findings. Abdom Imaging 36:174–178
5. Bennett GL, Rusinek H, Lisi V et al (2002) CT findings in acute gangrenous cholecystitis. AJR Am J Roentgenol 178:275–281
6. Piscaglia F, Nolsoe C, Dietrich CF et al (2011) The EFSUMB guidelines and recommendations on the clinical practice of contrast enhanced ultrasound (CEUS): update 2011 on non-hepatic applications. Ultraschall Med 33:33–59
7. Tang S, Wang Y (2013) Contrast-enhanced ultrasonography to diagnose gallbladder perforation. Am J Emerg Med 31:1240–1243
8. Yokoe M, Takada T, Strasberg SM et al (2012) New diagnostic criteria and severity assessment of acute cholecystitis in revised Tokyo guidelines. J Hepatobiliaty Pancreat Sci 19:578–585
9. Claudon M, Dietrich CF, Choi BI et al (2013) Guidelines and good clinical practice recommendations for contrast enhanced ultrasound (CEUS) in the liver—update 2012: a WFUMB-EFSUMB initiative in cooperation with representatives of AFSUMB, AIUM, ASUM, FLAUS and ICUS. Ultrasound Med Biol 39:187–210
10. Xu HX (2009) Contrast-enhanced ultrasound in the biliary system: potential uses and indications. World J Radiol 1:37–44
11. Derici H, Kara C, Bozdag AD, Nazli O, Tansug T, Akca E (2006) Diagnosis and treatment of gallbladder perforation. World J Gastroenterol 12:7832–7836
12. Bedirli A, Sakrak O, Sozuer EM, Kerek M, Guler I (2001) Factors effecting the complications in the natural history of acute cholecystitis. Hepatogastroenterology 48:1275–1278
13. Schafer M, Krahenbuhl L, Buchler MW (2001) Predictive factors for the type of surgery in acute cholecystitis. Am J Surg 182:291–297
14. Borzellino G, Steccanella F, Mantovani W, Genna M (2013) Predictive factors for the diagnosis of severe acute cholecystitis in an emergency setting. Surg Endosc 27:3388–3395
15. Contini S, Corradi D, Busi N, Alessandri L, Pezzarossa A, Scarpignato C (2004) Can gangrenous cholecystitis be prevented?: a plea against a "wait and see" attitude. J Clin Gastroenterol 38:710–716
16. Simeone JF, Brink JA, Mueller PR et al (1989) The sonographic diagnosis of acute gangrenous cholecystitis: importance of the Murphy sign. AJR Am J Roentgenol 152:289–290
17. Hunt DR, Chu FC (2000) Gangrenous cholecystitis in the laparoscopic era. Aust N Z J Surg 70:428–430
18. Teefey SA, Baron RL, Radke HM, Bigler SA (1991) Gangrenous cholecystitis: new observations on sonography. J Ultrasound Med 10:603–606
19. Kane RA (1980) Ultrasonographic diagnosis of gangrenous cholecystitis and empyema of the gallbladder. Radiology 134:191–194
20. Jeffrey RB, Laing FC, Wong W, Callen PW (1983) Gangrenous cholecystitis: diagnosis by ultrasound. Radiology 148:219–221
21. Chau WK, Na AT, Feng TT, Li YB (1988) Ultrasound diagnosis of perforation of the gallbladder: real-time application and the

demonstration of a new sonographic sign. J Clin Ultrasound 16:358–360
22. Sood BP, Kalra N, Gupta S et al (2002) Role of sonography in the diagnosis of gallbladder perforation. J Clin Ultrasound 30:270–274
23. Piscaglia F, Bolondi L (2006) The safety of Sonovue in abdominal applications: retrospective analysis of 23188 investigations. Ultrasound Med Biol 32:1369–1375
24. Kumagai Y, Kotanagi H, Ishida H et al (2006) Gallbladder adenoma: report of a case with emphasis on contrast-enhanced US findings. Abdom Imaging 31:449–452
25. Zheng SG, Xu HX, Liu LN et al (2013) Contrast-enhanced ultrasound versus conventional ultrasound in the diagnosis of polypoid lesion of gallbladder: a multi-center study of dynamic microvascularization. Clin Hemorheol Microcirc 55(3):359–374
26. Sparchez Z, Radu P (2012) Role of CEUS in the diagnosis of gallbladder disease. Med Ultrason 14(4):326–330
27. Xie XH, Xu HX, Xie XY et al (2010) Differential diagnosis between benign and malignant gallbladder diseases with real-time contrast-enhanced ultrasound. Eur Radiol 20(1):239–248
28. Liu LN, Xu HX, Lu MD et al (2012) Contrast-enhanced ultrasound in the diagnosis of gallbladder diseases: a multi-center experience. PLoS One 7(10):e48371
29. Inoue T, Kitano M, Kudo M et al (2007) Diagnosis of gallbladder diseases by contrast-anhanced phase-inversion harmonic ultrasonography. Ultrasound Med Biol 33:353–361
30. Adamietz B, Wenkel E, Uder M et al (2007) Contrast enhanced sonography of the gallbladder: a tool in the diagnosis of cholecystitis? Eur J Radiol 61:262–266
31. Zechner PM, Rienmuller S, Dorr K, Genger C, Wurzer H (2012) Contrast-enhanced ultrasound detects gallbladder perforation in a patient with acute abdominal pain. Am J Emerg Med 30(516):e515–e516
32. Yashima YTT, Nakahara S, Ishida K, Nakata R, Isayama H, Koike K (2011) Contrast-enhanced ultrasonographic image of gangrenous gallbladder. J Med Ultrason 38:239–241