Case Series

The impact of a rapid imaging protocol in acute cholecystitis—prospective cohort study

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A B S T R A C T

INTRODUCTION: In this study we assess the impact of a “rapid imaging protocol” (RIP) on outcomes in patients with suspected acute cholecystitis (AC).

METHODS: From January 2017 to January 2018, a prospective cohort study was implemented using a RIP with hepatoscintrigraphy (HIDA) or CT scan (first available, goal within 4 h) in patients (n=52) presenting with highly suspected AC and a clinical feature score of ≥1. For the latter, the following presenting features were scored as follows: 1 point for WBC count ≥10,000 (10^9/L), 1.5 points for glucose ≥140 (mg/dL), and/or 1 point for age ≥50 years. The historical control was all patients admitted with suspected AC in a 1.5-year period (n=117) under our previous “delayed imaging protocol” (DIP), which used US ± HIDA (post-admission) in select patients. Primary end points included: compare outcome and quality measures between the groups, evaluate diagnostic imaging performance for AC, and evaluate our proposed clinical feature score in the setting of AC.

RESULTS: Histopathologic features consistent with AC was more frequent in patients in the RIP (64% vs 39%, p=0.008). The pooled positive predictive value of HIDA and CT scan for AC were 85% vs 94%, respectively. The RIP was associated with a significant reduction in time to surgery, length of stay, and conversions to open (p<0.001, respectively). A clinical feature score of 3.5 predicted the likelihood of AC in 95% of the cases (χ^2 for linear trend=42, p<0.001).

CONCLUSION: A protocol centered around rapid identification, defined clinical criteria (i.e. clinical feature score), and confirmation with non-user dependent imaging modalities has resulted in favorable outcomes. CT may be the study of choice when the likelihood of AC is high because it is superior at identifying severity.

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1. Introduction

Acute cholecystitis (AC) requires prompt diagnosis to reduce perioperative morbidity and complications [1,2]. Modern guidelines recommend the diagnosis be made with a combination of clinical features, laboratory work up, and imaging confirmation [3,4]. For the latter, ultrasound has been the most commonly used diagnostic modality but performance in the setting of AC has been questioned by multiple reports [5,6]. Hepatoscintrigraphy (HIDA) scan is the gold standard imaging modality in AC, however, its use is limited to centers that have access. Computed tomography (CT) may have high diagnostic yield but few studies have evaluated the performance of CT in the setting of AC [7,8]. In this prospective study, we evaluate a rapid imaging protocol (RIP) using HIDA or CT in highly suspected AC and compare outcomes of this protocol with our previous protocol.

2. Methods

From January 2017 to January 2018 (one year), a prospective cohort study was implemented using a rapid imaging protocol (RIP) with HIDA or CT scan (first available, goal within 4 h) in patients (n=52) presenting with highly suspected AC. All patients presented to the emergency room (ER) and first contact was made by an ER physician. A high suspicion for AC was determined if the patient presented with progressive RUQ pain and/or positive Murphy’s sign, and a clinical feature score of ≥1. For the latter, the following presenting clinical features were scored as follows: 1 point for WBC...
count ≥ 10,000 (10⁹/L), 1.5 points for glucose ≥ 140 (mg/dL), and/or 1 point for age ≥ 50 yrs. Laboratory work up also included parameters to exclude other hepatobiliary pathologies (i.e. cholelithiasis, cholangitis, pancreatitis, hepatitis, etc.). If a high clinical suspicion remained after initial work up, the patient would then proceed with a HIDA or CT scan (first available, goal within 4 h), and the patient was managed accordingly (see Fig. 1). Some patients in the RIP received immediate ultrasound by the first contact ER physician in their preliminary work up, but if they met criteria for the study they were included and the RIP was followed accordingly. Most patients (overall, 50/52 cases) would eventually undergo laparoscopic or open cholecystectomy, and a final histopathologic diagnosis with our institutional pathologist. The operations were performed by the on-call/admitting surgeon (5 surgeon experience) with the assistance of a junior or senior general surgery resident in all cases. The content of this study has been reported in line with the PROCESS criteria [12].

For our historical control (i.e. “delayed imaging protocol,” DIP) we reviewed records of all patients that were admitted through ER in a 1.5-year period (January 2013 to July 2014) with a preliminary diagnosis of AC. During this time, the majority received an ultrasound in ER and select patients received a delayed HIDA post admission. All patients underwent cholecystectomy during the admission.

Primary end points included: 1) assess the performance of US, HIDA, and CT scan as imaging modalities in suspected AC, 2) compare outcome and quality measures (time to surgery, length of stay, costs, etc.) between the RIP vs DIP, and 3) evaluate our proposed clinical feature score in the setting of AC.

### 2.1. Clinical feature score

The features of the clinical feature score were determined by multivariate analysis of all presenting baseline clinical and laboratory findings in the historical group (n = 117). The details of this analysis have been previously described [9]. When all presenting features were analyzed, WBC count ≥ 10,000 (10⁹/L), glucose ≥ 140 (mg/dL), and age ≥ 50 years were the three presenting features that had a statically significant positive association with pathologically confirmed acute cholecystitis. Therefore, the clinical feature score was derived using a regression coefficient based scoring system from the multivariate regression analysis. Specifically, the regression coefficient (RC) for glucose ≥ 140 was 1.5 (yielding a score of 1.5), WBC count ≥ 10,000 and age ≥ 50 years both had an RC of 1 (yielding a score of 1), respectively.

#### 2.2. Imaging protocol

##### 2.2.1. Abdominal ultrasound (US)

All the abdominal US performed in this study were done prior to admission in the ER setting. Sonographic analysis was performed by a certified ultrasound technician and interpreted by a board-certified radiologist. All interpretations were done in real time and the original radiologic interpretation was not changed (i.e. the radiologist was not given the opportunity to carry out a second review for this study). The final US interpretation was determined as follows:

- Findings consistent with AC: sonographic Murphy’s sign, pericholecystic fluid, gallbladder distension (≥ 4 cm short axis), and/or thickening of the gallbladder wall (≥ 3 mm). Visualization of one of the four major signs was interpreted as positive for AC. If these one or more of these findings was not clearly identified the study was interpreted as negative for acute cholecystitis.
- Cholelithiasis: Visualization of none of the above four signs with gallstones.

##### 2.2.2. HIDA

A dynamic biliary study was performed following the intravenous administration of 6.1 mCi of Tc-99m Cholecyst (a hepatobiliary radiopharmaceutical agent). Sequential scintigraphic images of the abdomen in the anterior projection up to 60 min was done to evaluate for radiotracer distribution/activity throughout the hepatic parenchyma, intrahepatic ducts, gallbladder, common bile duct, and small bowel at 60 min. If no visualization of the gallbladder was present 1 h into the study, morphine (5 mg) was given intravenously to stimulate retrograde filling of the gallbladder. No visualization of the gallbladder at 30 min following morphine administration in the proper clinical setting, this scintigraphic find-
ing was reported to be consistent with acute cholecystitis. If there was delayed filling following administration of morphine in the proper setting, this scintigraphic finding was reported to be consistent with chronic cholecystitis.

2.2.3. CT scan

An abdominal CT scan without contrast was obtained using helical technique via a Philips Diamond Select Brilliance CT 64-slice (Philips Medical Systems, Best, The Netherlands). The standard slice thickness was 5 mm. Most CT scans were reviewed by at least two board certified radiologists, and in all cases the real time interpretation was used (i.e. a second look was not offered for our study) and the radiologist was blinded to the study.

Findings consistent with AC included: gallbladder distention (≥4 cm short axis), gallbladder wall thickening (≥3 mm), pericholecystic fluid, pericholecystic fat stranding, focally increased enhancement of adjacent liver parenchyma, pericholecystic abscess, irregular contour or enhancement of the gallbladder wall, and/or gas in the wall or lumen. If one or more of these findings was not clearly identified the study was interpreted as negative for acute cholecystitis.

2.3. Determining diagnostic performance (all imaging modalities)

A patient was classified as true-positive (TP) if the diagnosis of AC was retained on the final pathology report and the sign or the association of signs on the imaging test was indicative of AC. A patient was classified as true-negative (TN) if the diagnosis of AC was not retained and the sign or the association of signs was not indicative of AC. A patient was classified as false-positive (FP) if the diagnosis of AC was not retained but the sign or the association of signs was indicative of AC. A patient was classified as false-negative (FN) if the diagnosis of AC was retained but the sign or the association of signs was not indicative of AC (see Fig. 2). The sensitivity was defined as TP/(TP+FN) and the specificity was defined as TN/(FP+TN). The positive likelihood ratio was defined as sensitivity/1-specificity and the negative likelihood ratio was defined as 1-sensitivity/specificity. The disease prevalence was defined as TP+FN/(TP+FN+FP+TN). The positive predictive value was defined as TP/(TP+FP) and the negative predictive value was defined as TN/(FN+TN). Accuracy was defined as TP+TN/(TP+FN+FP+TN).

2.4. Pathological diagnosis

The histopathological criteria used in the analysis of gallbladder specimens was as follows:

- Absence of cholecystitis: normal gallbladder and/or absence of lymphocytic infiltration.
- Acute cholecystitis (AC): transmural neutrophilic infiltration ± involvement of adventitia (pericholecystitis) ± gangrene.
- Chronic cholecystitis (CC): lymphocytic infiltrates without neutrophilic infiltrates.
- Acute superimposed on chronic cholecystis (ACC): the presence of acute and chronic features as noted above.
- Acalculous Cholecystitis: AC or ACC without gallstones

2.5. Cost analysis

A cost analysis was carried out by identifying mean payments made by the Puerto Rico Medicaid program (the largest payer system in Puerto Rico) to our institution as follows: 1) a bundled payment for each inpatient day for diagnosis of AC and coverage of medications ($630.00), 2) US ($36.67), 3) HIDA ($97.95), and CT ($75).

2.6. Statistical analysis

Statistical analysis was performed using the IBM SPSS version 21 (IBM Co., Armonk, NY, USA). Continuous data are reported as means. Nominal data are reported as percentages and/or number of subjects. Comparisons between groups were analyzed using either Student’s t test or cross table analysis accordingly. The chi square (x²) test of linear trend was used to test association between nominal and ordinal variables.

Using our length of stay (LOS) data in the DIP (n = 117, mean LOS = 5 days, SD = 3.1) to determine power, the analysis indicated that in order to detect a mean LOS of 3 days (our pre-study goal) in the RIP, a total of 29 subjects were needed for 90% power and a type 1 error rate of <0.05.

3. Results

3.1. Historical results

In our historical group, histopathologic features consistent with AC were present in 46/117 (39%) specimens. US had a sensitivity and specificity of 26% and 80%, respectively. HIDA scan had a sensitivity and specificity of 87% and 78%, respectively. Time to surgery (TTS) was 4 vs 2 days in patients who received HIDA vs US alone (p < 0.001), and LOS was 6 vs 4 days, respectively (p = 0.001). Age ≥ 50 years, glucose ≥ 140 mg/dL, and WBC count ≥ 10 (10⁶/L) were statistically significant independent variables associated pathologically confirmed AC.

3.2. Baseline characteristics

In the RIP, a total of 52 patients presented to ER with suspected AC and were admitted to the surgery service for further management. In the DIP, a total of 117 patients presented to ER with suspected AC and were admitted for further management. Woman were the most commonly affected gender in both groups, representing 62% vs 66% (RIP vs DIP, p = 0.52), respectively. The mean age was 50 vs 44 years old (RIP vs DIP), and there was a trend that patients in the RIP were older (p = 0.07). The mean BMI (body mass index, kg/m²) for both groups was 30 and there was no statistical difference between the groups for comorbidities (i.e. hypertension, coronary artery disease, diabetes, and/or hyperlipidemia). There was no difference between the groups for basic and hepatobiliary laboratory parameters (see Table 1) except hyperglycemia (glucose ≥ 140 mg/dL) which was more frequent in the RIP.

| Table 1 Baseline characteristics and laboratory results. *Clinical feature score = glucose ≥ 140 (1.5 points), WBC count ≥ 10,000 (1 point), age ≥ 50 yrs. (1 point). The higher the score the higher the likelihood of acute cholecystitis. |
|-----------------|-----------------|-----------------|----------------|
| Baseline characteristics/labs | RIP | Delayed | p-value |
| Age (years) | 50 | 44 | 0.07 |
| Sex (F/M) | 32/20 | 77/40 | 0.58 |
| BMI (kg/m²) | 30 | 30 | 0.58 |
| WBC (>10⁶/L) | 13 | 12 | 0.27 |
| Glucose >140 mg/dL | 37% | 15% | 0.002 |
| AST (U/L) | 50 | 56 | 0.72 |
| ALT (U/L) | 51 | 82 | 0.07 |
| Amylase (U/L) | 76 | 57 | 0.38 |
| Lipase (U/L) | 160 | 150 | 0.54 |
| Total bilirubin (mg/dL) | 0.69 | 0.83 | 0.24 |
| Direct bilirubin (mg/dL) | 0.21 | 0.28 | 0.43 |
| ALP (U/L) | 106 | 117 | 0.48 |
| Clinical feature score* | 1.8 | 1.1 | 0.001 |

Note: *Clinical feature score = glucose ≥ 140 (1.5 points), WBC count ≥ 10,000 (1 point), age ≥ 50 yrs. (1 point). The higher the score the higher the likelihood of acute cholecystitis.
Overall, patients in RIP had a higher clinical feature score (1.8 vs 1.1, p = 0.001), and more patients had at least one clinical feature (98% vs 68%, p = 0.001).

3.3. Operative outcomes

Overall, 167/169 patients underwent cholecystectomy. In the RIP, laparoscopic cholecystectomy was performed in 49/52 (94%) and one planned open cholecystectomy. Two patients were treated with cholecystostomy tube. In the DIP, laparoscopic cholecystectomy was performed in 110/117 (94%) and six patients were treated with a planned open cholecystectomy. No patients in the DIP were treated with cholecystostomy tube. There were no intraoperative conversions to open technique in the RIP, which was statistically significant when compared to the DIP (0% vs 12%, p = 0.01). Time to surgery was significantly lower in the RIP (1.1 vs 2.8 days, p = 0.001).

3.4. Histopathologic outcomes

Histopathologic features consistent with acute cholecystitis (AC) was more frequent in patients in the RIP (64% vs 39%, p = 0.008). In the RIP, acute superimposed on chronic cholecystitis (ACC) with gallstones (20/50, 40%) was the most common final pathology, and ACC with gallstones (12/50, 24%) was the second most common pathology (see Fig. 3). In the DIP, chronic cholecystitis (CC) with gallstones (52/177, 44%) was the most common pathology, and ACC with gallstones was the second most common pathology (21/117, 18%). Overall, gallstones were found in 134/167 (80%) of all pathologic specimens.

3.5. Ultrasonography performance for AC

Overall, a total of 134 US studies were performed prior to cholecystectomy. Twenty-eight were in the RIP and 106 were in the DIP. The sensitivity and specificity of US in the RIP patients was 47% and 82%, respectively. The pooled sensitivity, specificity, positive and negative predictive likelihood ratios of US were 32, 80, 1.61, 0.85, respectively (see Table 2). The pooled positive predictive value was 56%. There were 19 true positive (TP), 60 true negative (TN), 40 false negatives (FN), and 15 false positives (FP) interpretations, respectively. ACC + S was the final pathology in 23/40 (58%) cases that were FN on US.

3.6. HIDA performance for AC

Overall, a total of 55 HIDA studies were performed prior to cholecystectomy. Twenty-one were in the RIP and 34 were in the DIP. The sensitivity and specificity of HIDA in the RIP patients was 83% and 100%, respectively. The pooled sensitivity, specificity, positive and negative predictive likelihood ratios of HIDA were 85, 86, 6, 0.17, respectively (see Table 2). The pooled positive predictive value was 85%. There were 23 TP, 24 TN, 4 FN, and 4 FP interpretations, respectively. ACC + S was the final pathology in 3/4 (75%) cases that were FN on HIDA.

3.7. CT performance for AC

Overall, a total of 39 CT studies were performed prior to cholecystectomy. All patients who received CT were in the RIP. The pooled sensitivity, specificity, positive and negative predictive likelihood ratios of CT were 73, 94, 12, 0.30, respectively (see Table 2). The positive predictive value was 94%. There were 16 TP, 16 TN, 6 FN, and 1 FP interpretations, respectively. ACC + S was the final pathology in 4/6 (67%) cases that were FN on CT.

3.8. Perioperative data and quality measures

Overall, there was no significant difference between RIP vs DIP for postoperative complications (2 vs 6, p = 0.55). One patient in the RIP developed a biliary leak after laparoscopic cholecystectomy, which was treated with ERCP/stent. Two patients in the DIP developed biliary leak after laparoscopic cholecystectomy and they were treated with ERCP and stent. One patient in the RIP developed symptomatic anemia (post op Hgb = 7.4) related to hemorrhage from the gallbladder fossa dissection (a drain was left inopera-
tively) after laparoscopic cholecystectomy, which was successfully treated with 2 packed red blood cell units. Two patients in the DIP developed postoperative hypoxia after open cholecystectomy and were found to have small right sided pleural effusions in both cases. The latter were treated with aggressive pulmonary toilet and did not require any further intervention. One patient in DIP developed a superficial skin surgical site infection, which was treated with drainage and IV antibiotics. There was no postoperative mortality in either group. Admission to surgery and length of stay were significantly shorter in the RIP protocol (p = 0.001, respectively). Surgery to discharge time was also significantly reduced in the RIP (1.9 vs 3.1 days, p = 0.001). The estimated cost to payer was reduced by 35% in the RIP when compared to DIP (see Table 3).

3.9. Validation of the clinical feature score

An increasing clinical feature score had a strong association for likelihood of AC (χ² for linear trend = 42, p<0.001). Overall, 20/21 (95%) patients with a score of 3.5 had a final pathology result consistent with acute cholecystitis (see Table 4). A clinical score of at least 2 was found in 24/31 (77%) patients with pathologically confirmed gangrene, and an increasing clinical score had a strong association with progression to gangrene (χ² for linear trend = 27, p<0.001).

4. Discussion

Modern protocols in the setting of AC are geared to reliably diagnose and assess the severity of disease on presentation [1,2]. While most patients will ultimately require an urgent cholecystectomy, some patients may be better served with a tailored approach. We designed our RIP after a thorough review of our historical data, in which we looked at the diagnostic performance of ultrasound for AC, perioperative data, and quality outcomes. For the latter study, we found that not only was US underperforming (sensitivity 26% for AC) but our classic approach was associated with delayed treatment [9]. However, the latter findings could not be attributed to poor management. Instead, we found that acute cholecystitis presented with typical and atypical features, as well as variable severity which can delay identification. Chronic cholecystitis was frequent in our population and common in patients that progressed to true AC (i.e. sustained irreversible obstruction of the cystic duct with down stream mucosal ischemia and necrosis). Moreover, most patients with CC reported symptoms consistent with a short-lived AC episode which had reversed with self-treated NPO status, in many cases multiple times when pressed on history. Thus, acute superimposed on chronic cholecystitis represents the most common subtype of AC in our population and classic ultrasound findings for AC are often masked, leading to false negative imaging interpretations. Overall, only 38/167 (23%) of patients presented with de novo AC (i.e. AC not associated with CC), which in general are usually easier to identify clinically and with any common imaging modality.

Our RIP is centered around the principles reported by the Tokyo guidelines [3,4] as follows: 1) high clinical suspicion and features, 2) key laboratory findings (i.e. leukocytosis, inflammatory markers, etc.), and 3) confirmatory imaging. Clinical suspicion is based on history and physical examination (most patients will have epigas-
tric/RUQ pain). In our experience Murphy’s is a positive predictor of AC, however, lack of this sign does not exclude AC. For this reason, further work up is needed to confirm and assess severity of disease. We have found that age greater than 50 is a clinical feature that should increase suspicion, as this feature had a statistically significant independent association with pathologically confirmed AC. Once a high index of clinical suspicion is determined, a full set of labs is needed to exclude other hepatobiliary disease, most importantly pancreatitis and cholangitis. These must be excluded because management of these cases must be tailored accordingly. Once the latter are excluded, glucose and leukocytosis correlate independently for AC. Moreover, if both features are present, our data suggests a 70% likelihood of AC in the proper setting. Many experts have recommended inflammatory markers (i.e. c-reactive peptide) to get an indirect assessment of severity [3,4], however, we did not routinely include this in our RIP. Nonetheless, our data demonstrates that our clinical feature score is not only predictive for AC, progression to advanced cholecystitis (i.e. gangrenous, emphysematous, etc.) was frequent in patients with two or more features.

Imaging has historically been centered around US despite studies that have shown that it underperforms in the setting of AC [5,6]. Based on the latter studies, and our own experience with US, we designed the RIP centered around confirmation with non-user dependent studies (i.e. HIDA and CT scan). The former was implemented because it is generally accepted that HIDA is the gold standard test to confirm AC and it is readily available in our institution. The CT scan was implemented because it is readily available in most institutions and the few studies using this modality have shown promising diagnostic performance in the setting of AC. We found that both studies have similar diagnostic performance in the setting of de novo AC (i.e. without chronic pathologic features), however, both studies are affected in the setting of ACC (i.e. acute superimposed on chronic). For the latter, HIDA is superior to CT and US, however, the false negative (FN) HIDA interpretations in the RIP (n = 3) were all in the setting of ACC. Overall, 3/4 (75%) FN HIDAs had delayed filling which required morphine to fill the gallbladder, which was interpreted by our nuclear specialist as chronic cholecystitis. Therefore, we recommend that in a patient with intermediate risk (i.e. clinical feature score 1–2, or 20–60% pretest likelihood of AC) and delayed filling on HIDA, the diagnosis is ACC until proven otherwise and should be managed with urgent cholecystectomy. CT scan was non-inferior in the setting of de novo AC, especially in high risk patients (i.e. clinical feature score ≥2.5 or ≥70% pretest likelihood of AC). A high clinical score correlated with advanced AC, and a CT scan was the ideal imaging study to assess the severity of disease (i.e. gangrene, perforation, abscess, etc.). Moreover, a CT scan was helpful for planning the intervention approach based on severity (i.e. laparoscopic vs open vs percutaneous drainage).

The RIP protocol resulted in improved outcomes when compared to the DIP. Identification and treatment were hastened, perioperative morbidity/complications were significantly reduced, and quality measures/costs reduced accordingly. Rapid identification and allocation were the main reasons for these better outcomes, which is consistent with reports advocating early (<72 h) cholecystectomy [10,11]. Delayed diagnosis results in an advanced inflammatory process with unfavorable anatomy if cholecystectomy is pursued. In our RIP we have had no conversions to open due to unfavorable anatomy, which suggest early cholecystectomy is associated with a more technically feasible procedure regardless of pathologic grade. There was one biliary leak post op in the RIP which was successfully managed with ERCP and temporary stent. However, the latter patient presented to ER with gangrenous cholecystitis in severe sepsis 7 days after onset of symptoms, and required resuscitation and broad-spectrum antibiotics for 2 days in ICU prior to cholecystectomy. Therefore, the patient was high risk for developing perioperative complications due to delay in management.

The current study has some limitations. The patients were all drawn from a primarily Hispanic population which lacks the random distribution of prospective population-based study designs. Moreover, restriction of inclusion criteria to patients fulfilling diagnostic criteria (i.e. at least one clinical feature score) may have resulted in a selection of patients with more advanced AC, thus, mild disease stages may be under represented in the present study. Despite these limitations, our academically driven natural history study defines the spectrum of disease of AC in Puerto Rico, a population with an epidemic of obesity and associated conditions. Our data suggest that mild, moderate, and severe forms of AC exist. Moreover, acute superimposed on chronic cholecystitis has particular clinical and diagnostic considerations, which were frequently encountered in our cohort. Future studies are needed to determine which imaging modality is best in each grade. Our study is also one of the first to our knowledge to prospectively apply a clinical feature score in the setting of AC, which we used longitudinally in our RIP cohort.

In closing, AC is a pathology that requires prompt and precise diagnosis. ACC is the most common subtype of AC in our population and is associated with false negative imaging interpretations related to masking of the typical imaging features (edema, pericholecystic fluid, etc.) that manifest in de-novo cholecystitis. HIDA scan is ideal in intermediate risk cases where the diagnosis is equivocal and severity (i.e. gangrene, necrosis, etc.) is not a concern. CT scan is ideal when there is a high likelihood for AC and severity is a concern. A protocol centered around rapid identification, defined clinical criteria (i.e. clinical feature score), and confirmation with non-user dependent imaging modalities has resulted in favorable outcomes. When these principles are used in conjunction, management of each patient is tailored accordingly to maximize efficiency and safety of treatment for all comers with suspected AC.

Conflicts of interest

None.

Sources of funding

None.

Ethical approval

This study was approved by the Ponce Health Sciences University IRB.

Consent

Consents for use of deidentified information for research and publication was attained for all patients.

Author contributions

Limael E. Rodriguez—primary responsibility.
Jorge A. Sanchez—study concept or design, data collection, data analysis or interpretation.
Miguel A. Serpa—study concept or design, data analysis.
Jorge L. Martinez—study concept or design, data analysis.
Julio A. Peguero-Rivera—study concept or design, data analysis.
Felipe Sanchez-Gaetaen—study concept or design, data analysis.
Guillermo Bolanos-Avila—study concept or design, data analysis.
Registration of research studies

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Guarantor

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