



## Does a Negative Triple Test Reduce the Rate of Negative Appendectomy in Adults?

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### Abstract

**Objectives:** To investigate the negative predictive value (NPV) of the triple test (total leukocyte count [TLC], neutrophil percentage [NP], and C-reactive protein [CRP] level) in the diagnostic accuracy of acute appendicitis by reducing negative appendectomy rates (NARs) in adult patients.

**Methods:** The present prospective clinical study included 348 consecutive patients admitted to Ahmadi Hospital, Kuwait during 2010 and 2011 with the possible diagnosis of acute appendicitis. Children (<16 years) (n=61) and pregnant women (n=5) were excluded. The remaining 282 patients were enrolled but only those with an NTT (n=102) were analyzed (study population). The NTT meant TLC <11,000/ $\mu$ L, NP <75%, and CRP <5 mg/L. The data collected included demographics, clinical presentation, laboratory tests, histopathology, and outcome, and the NPV was calculated.

**Results:** Sixty-seven patients (65.9%) were female and 35 (34.1%) were male. Their ages ranged between 16 and 49 years (mean 27.5 years). Most patients (87.3%) had their symptoms for 12-36 hours before hospital admission. The mean values for TLC, NP, and CRP were 7,573/ $\mu$ L, 54.53%, and 0.61 mg/L, respectively. Of the 102 patients with NTT, 101 (99%) proved not to have appendiceal inflammation (NPV=99%). Only 39 patients were operated upon, of whom 38 (97.4%) had a normal appendix, and the remaining 63 patients were either discharged (n=47) or referred to other specialties (n=16). There were significantly more women (76.3%, 29/38) with negative appendectomy than men (24.7%, 9/38) ( $\chi^2=21.1$ ,  $p=0.0001$ ). Gynecological causes were the most common (60.5%, 23/38) and in 11 cases, the exact etiology could not be identified.

**Conclusions:** From the data presented, it may be concluded that TLC, NP and CRP blood levels (triple test) should be measured upon hospital admission of adult patients with clinically suspected acute appendicitis. If used judiciously, they may spare the group of patients with an NTT an unnecessary surgical operation, hence markedly reducing the NAR with its potential risks.

**Key words:** Acute appendicitis, leukocytic count, neutrophils, C-reactive protein, triple test

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### Introduction

The diagnosis of acute appendicitis is predominantly a clinical one. However, it can be challenging even in the most experienced of clinical hands because of the many different conditions that manifest

with acute abdominal pain as well as the relatively non-specific initial presentation of the disease [1]. Compounding the diagnostic challenge, no single sign, symptom, or diagnostic test confirms the diagnosis of appendiceal inflammation in all cases.

Because the natural history of appendicitis is time-dependent, complications, such as perforation most of the time, accurate diagnosis and a timely operation, are important to prevent unnecessary surgery and to avoid complications [2,3].

Appendectomy is by far the commonest major emergency general surgical operation [4] (4). Based on unaided clinical diagnosis, the negative appendectomy rate (NAR) is about 15-30% and reaches up to 30-50% in women of a childbearing age because of the prevalence of gynecological diseases [5]. These figures are no longer accepted, and ought to be reduced by supplementary measures [6], since a negative appendectomy (NA) carries some risks for the patient, including adhesion formation, infection, post-operative disability, and may even require future surgery [7]. Methods advocated to assist in the diagnosis of appendicitis include inflammatory markers [8] (8), scoring systems [9,10], computer programs [11], ultrasonography (US) [12], computed tomography (CT) [13], magnetic resonance imaging (MRI) [14], scintigraphy [15], and diagnostic laparoscopy (DL) [16,17].

The present study was conducted to investigate the role of simple laboratory inflammatory markers, name-

ly total leukocyte count (TLC), neutrophil percentage (NP), and C-reactive protein (CRP) combined together (triple test), in identifying a group of patients with clinically suspected acute appendicitis in which unnecessary appendectomy could be avoided.

### Subjects and Methods

The present prospective, blind clinical study included 348 consecutive patients suspected of having acute appendicitis, who attended the Surgical Division, Ahmadi Hospital (Secondary Care Hospital), Kuwait during the years 2010 and 2011. Children (<16 years) (n=61) and pregnant women (n=5) were excluded from the study. The remaining 282 patients were enrolled but only those with the negative triple test (NTT) (n=102, 36.2%) were analyzed (study population). A triple test was considered negative if the CRP value was <5 mg/L, TLC <11,000/ $\mu$ L, and NP <75%. It was considered positive if any of the three tests was raised above the aforementioned figures (n=180, 63.8%). The study protocol was approved by the Ethical Committee of the hospital and all patients signed an informed written consent before enrollment in the study. Patient disposition is shown in Figure 1.

Clinical findings, including history of anorexia, pain

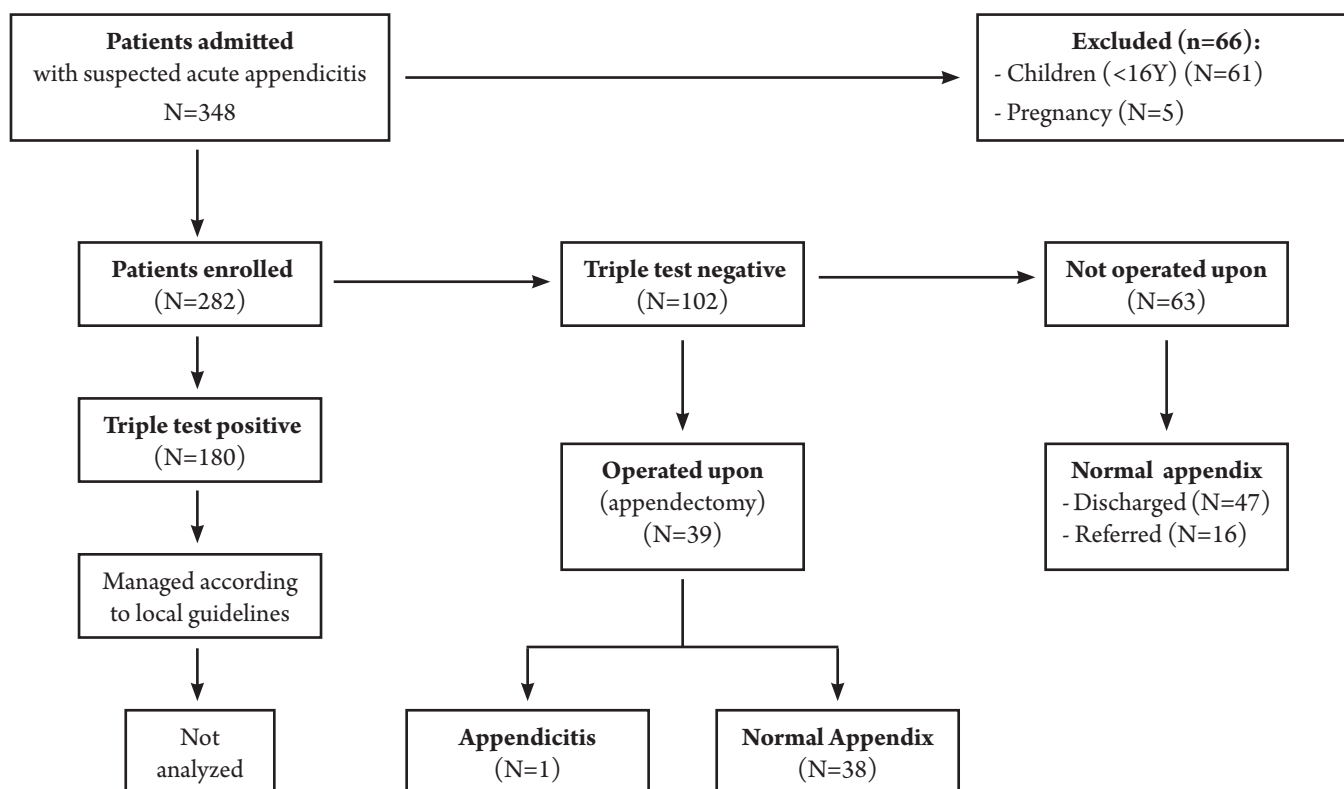


Figure 1. Patient flow chart.

followed by nausea, right lower quadrant (RLQ) pain, vomiting, diarrhea, fever, localized tenderness, guarding, rigidity, rebound tenderness (Blumberg sign) and Rovsing sign, were recorded for all patients and were evaluated with an active in-hospital observation. Duration of symptoms before hospital admission was documented. The decision to operate or not was made by the “blinded” surgical consultant, and was based primarily on clinical grounds and not influenced by the pre-operative levels of the triple test. Urine analysis and a triple test (TLC, NP, and CRP) were ordered for all patients upon admission. A pregnancy test was done routinely for all women in the childbearing period (18-40 years). The rapid immunometric method was used for quantitative estimation of CRP [18]. The results of the triple test were correlated with the histological findings that were graded as normal, simple inflamed, gangrenous and/or perforated. The negative predictive value (NPV) of the triple test was then calculated. Other diagnostic aids, such as US, CT or DL, were resorted to in selected cases. Statistical analysis was performed using the SPSS/PC version 15 (Prentice Hall, Chicago, IL). The Chi square test with Yate’s correction was used to compare proportions. The significance level was set at 5%.

### Results

The total number of patients studied was 102, of whom 67 patients (65.9%) were female and 35 (34.1%) were male, with a female-to-male ratio of 1.9:1. Their ages ranged between 16 and 49 years with a mean age of 27.5 years. All patients had lower abdominal pain, most of them (94.1%) had localized tenderness, and about two thirds had guarding, rigidity or rebound

tenderness (Table 1). The majority of the patients had their symptoms for 12-36 hours before hospital admission (89/102, 87.3%). Three patients (2.9%) only had their symptoms for 36-48 hours (Table 2). The mean TLC was 7,573/ $\mu$ L (range 2,943-10,720/ $\mu$ L), while the mean NP was 54.53% (range 13.92-73.85). The value of CRP ranged between 0 and 4.5 mg/L, with a mean of 0.61 mg/L.

**Table 2.** Duration of symptoms before hospital admission and NPV in the study population (n=102).

Duration of Symptoms	N (%)	NPV
< 12 hours	10 (9.8)	100.0%
12-24 hours	68 (66.7)	100.0%
24-36 hours	21 (20.6)	100.0%
36-48 hours	3 (3.9)	66.7%
<b>Total</b>	<b>102 (100.0)</b>	<b>99%</b>

As seen in Table 3, 99% (101/102) of the patients clinically suspected to have acute appendicitis as well as an NTT proved not to have appendiceal inflammation, yielding a NPV of 99%. Of those 102 patients, 63 (61.8%) were not operated upon and were either discharged from hospital because of complete clinical relief (n=47), or were further investigated with US, CT or DL and referred accordingly to other specialties (n=16), most commonly gynecology (10/16, 62.5%) and urology (4/16, 25%). Of the 39 patients who underwent appendectomy (30 women and 9 men), 38

**Table 3.** Outcome of negative triple test patients (n=102).

Outcome	N	%
<b>Not appendicitis (True negative test)</b>	<b>101</b>	<b>99</b>
Not operated upon	63	61.8
1. Discharged	47	
2. Referred to:	16	
• Gynecology (Ovarian disease) (n=10)		
• Urology (UTI) (n=4)		
• Internal Medicine: (IBD) (n=2)		
Operated upon (Normal, i.e. negative appendectomy)	38	37.2
<b>Appendicitis (False negative test) by histopathology:</b>	<b>1</b>	<b>1</b>
Simple inflamed	1	
Perforated / gangrenous	0	
<b>Total</b>	<b>102</b>	<b>100.0</b>

UTI: Urinary tract infection, IBD: Inflammatory bowel disease.

**Table 1.** Clinical findings in the study population (n=102).

Clinical Finding	N	%
Lower abdominal pain	102	100
Low-grade fever	27	26,5
Nausea and/or vomiting	30	29,4
Diarrhea	18	17,6
Localized tenderness	96	94,1
Guarding	71	69,6
Rigidity	65	63,7
Rebound tenderness	69	67,6
Rovsing sign	19	18,6

(97.4%) had an NA. Only a 26-year-old lady with a 40-hour duration of symptoms (2.6%, 1/39) had simple appendicitis, as reported by histopathology (false NNT). Of the 38 patients with NA, there were significantly more female patients (76.3%, 29/38) than males (24.7%, 9/38) ( $X^2=21.1$ ,  $p=0.0001$ ). The diagnosis of the 38 patients with NA is shown in Table 4. As may be seen, gynecological causes were the most common (60.5%, 23/38). In 11 cases (6 females and 5 males), the exact etiology could not be identified and patients were labeled to have had non-specific abdominal pain.

**Table 4.** Diagnosis in patients with negative appendectomy according to pathology (n=38).

Diagnosis	N	%
Ovarian cyst (not complicated)	9	23.7
Ruptured ovarian cyst	6	15.7
Twisted ovarian cyst	5	13.1
Endometriosis	3	7.9
Non-specific mesenteric adenitis	2	5.3
Crohn's disease	2	5.3
Non-identifiable cause (non-specific abdominal pain)	11	29.0
<b>Total</b>	<b>38</b>	<b>100.0</b>

## Discussion

Diagnosis of acute appendicitis relies largely on clinical assessment; however, the majority of patients present with non-specific symptoms, such as right lower quadrant (RLQ) abdominal pain, nausea and vomiting. In fact, any acute abdominal condition can mimic appendicitis and thus the list of differential diagnosis is long. Localized tenderness and evidence of peritoneal inflammation help in reaching a possible diagnosis. However, the NAR is still relatively high and has recently become non-acceptable. Laboratory investigations usually contribute a little and can be misleading [19]. Although appendectomy is considered to be a safe procedure, it is not without complications, which may include surgical site infection, an intra-abdominal abscess, adhesive bowel obstruction, and pulmonary complications, in addition to the risks of anesthesia. Appendectomy for a normal appendix is known to be associated with both mortality and morbidity [6]. Several authors have reported that the risk of intestinal obstruction following surgery for a normal appendix is

higher than that for a non-perforated inflamed appendix [20,21]. Styruud et al. [22], in their study of 2,351 patients undergoing appendectomy, reported that the risk of intestinal obstruction is increased by 5% in patients with a healthy appendix. Additionally, some patients have persistent symptoms after surgery, and constitute a burden on the hospital resources, while being unsatisfied with the health care provided.

Despite modern imaging techniques, NARs are still a problem for surgeons [23]. Over the last years, many studies have thus looked at various simple blood tests and clinical criteria in an attempt to improve diagnostic accuracy. Serum inflammatory markers, namely TLC and CRP, are now often used to guide the clinical assessment in reaching the proper diagnosis. The increase in TLC (leukocytosis) is an early sign of appendiceal inflammation, but with a sensitivity of 42%-96% and a specificity of 53%-76% [2,3,24]. Similarly, CRP, which increases with inflammation, has a sensitivity of 40%-96.6% and a specificity of 38%-94% [25-27]. This distractingly wide range of sensitivity and specificity is at least partly due to variations in the cut-off values and the differences in study populations [28]. Measurements of TLC or CRP alone have not been shown to be effective in preventing negative appendectomies, as they cannot distinguish between sites of infection or inflammation, and are therefore unlikely to predict a specific diagnosis, such as acute appendicitis, even when both TLC and CRP are raised [29]. Furthermore, Singupta et al. [30], contrary to other reports [31-33], showed that levels of TLC and CRP could not predict the development of complications, such as gangrene or perforation.

In the present study, we did not include children and pregnant women because children may not mount a normal response to infection, which renders inflammatory markers unreliable, and in pregnant women, the physiologic leukocytosis renders the TLC useless for the diagnosis of appendicitis. We also restricted the study population to those patients with an NTT. In other words, we particularly studied the value of the triple test, not in aiding the diagnosis of acute appendicitis, but rather in helping the clinician to rule out the diagnosis of appendicitis, thus reducing the NAR with certainty and safety. All the three tests (triple test) are



simple, inexpensive, rapid, and widely available blood tests.

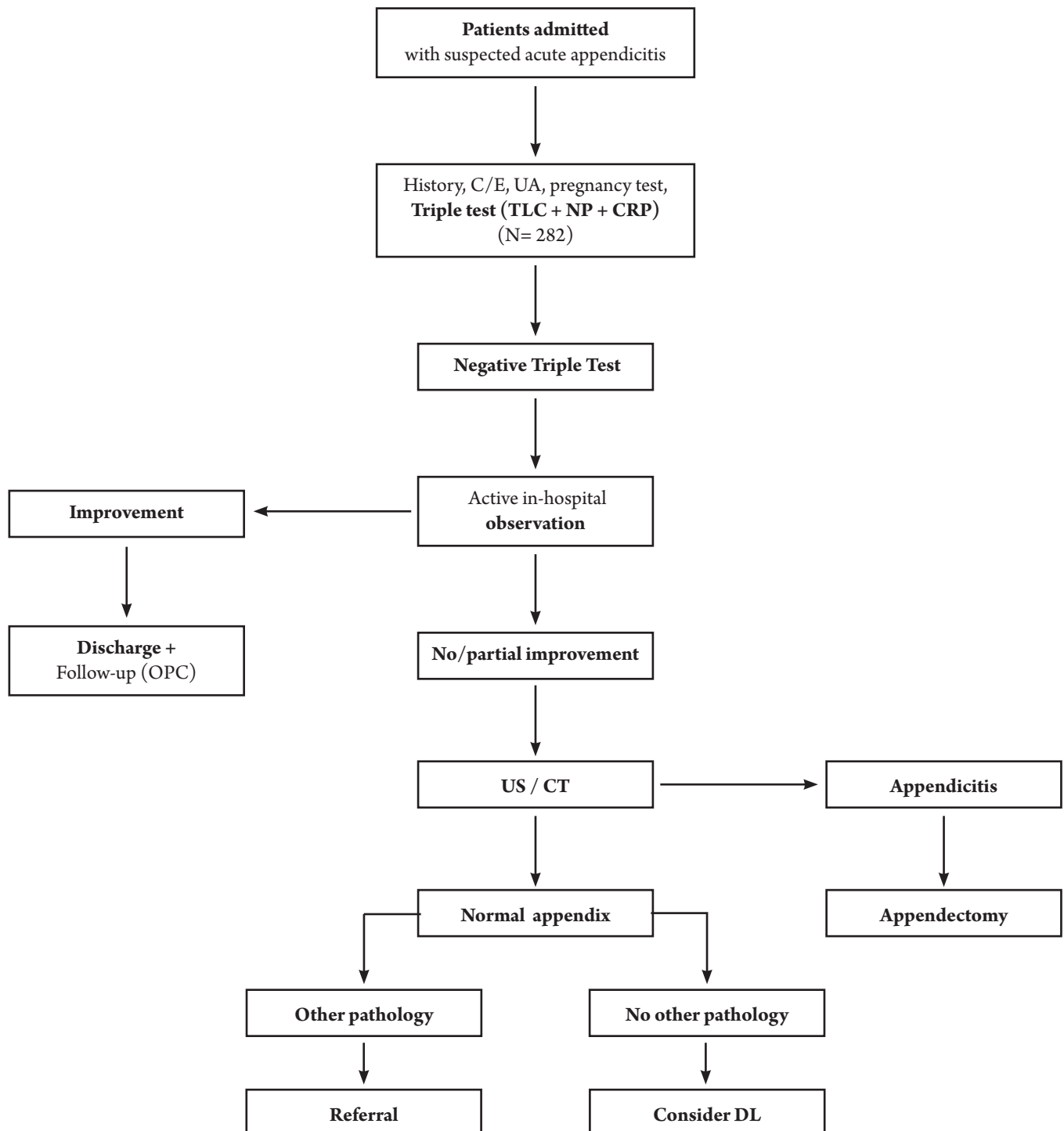
As opposed to other reports in the literature [30,34], women constituted about two thirds of the population in this study, which may be explained by the restriction of the study to those with an NTT, as well as the multiple gynecologic diseases that may mimic appendicitis without raising the inflammatory markers. Consequently, significantly more women had negative appendectomies. Similar results were reported by Singhal and Jadhav [4]. In their series of 199 appendectomies, 48.2% of female patients had a normal appendix, as opposed to only 18.8% among male patients.

Some authors [35,36] reported that when the CRP value is normal in a patient who has had his symptoms for more than 12 hours, this patient does not have acute appendicitis and can be followed in an outpatient setting. In the present study, however, the CRP value was used simultaneously with TLC and NP. An "NTT" was valuable in ruling out appendicitis even when patients were presenting with symptoms for less than 12 hours. The only patient who had appendicitis, despite an NTT, was a 26-year-old lady who presented with symptoms of 36-48 hours duration, but the small number of this sub-group of patients (n=3) does not permit any conclusions.

The most important finding of this study is the 99% NPV of the triple test, i.e. only one patient out of 102 had acute appendicitis when all three tests (TLC, NP and CRP) were normal. It is possible that some patients may have had early and self-limiting appendicitis that resolved spontaneously. However, irrespective of this possibility, they did not need appendectomy. If the negative results of these tests were put into consideration along with the clinical criteria, patients suspected of having acute appendicitis could have been put under active in-hospital observation without the fear of developing perforation, and in our study, 38 patients out of 39 could have been spared a negative appendectomy with its possible risks [6], and may not have even required admission to hospital [30]. The risk of appendiceal perforation has been reported to be related to the duration of symptoms before hospital admission, and not on the duration of in-hospital observation(37). In accordance with our findings, Yang et al. [37] found

that only 6 of 740 patients with appendicitis had normal TLC, NP and CRP, yielding a sensitivity of 99.2% for the triple screen, and concluded that acute appendicitis is very unlikely when the three tests are simultaneously normal. Moreover, Dueholm et al. [38] in their prospective blinded study of 204 patients, reported that the triple-test combination has an NPV of 100%, indicating that if the test is negative, acute appendicitis is unlikely. It can cut the NARs. Furthermore, Gronroos [39], in 2011, reported that the clinical suspicion of acute appendicitis can be excluded in adult patients if both TLC and CRP were normal on admission to hospital and remained normal in the follow-up. On the other hand, Vaughan-Shaw et al. [28], in 2011, reported their results of a retrospective cohort study of consecutive patients from 2 district general hospitals and reported a sensitivity of 92% and 94%. They suggested that patients with NTT should not be discharged home if there is high clinical suspicion of appendicitis, and they considered this strategy not risk-free.

Obviously, the assessment of patients with acute abdominal pain, and particularly suspected appendicitis, remains multifactorial, and still relies, to a large extent, on clinical judgment. In some patients, further investigations, such as US, CT and even DL, may be indicated, whereas in others a short period of observation is all that is required, before the diagnosis becomes clearer. On the basis of the results from this study, we suggest an algorithm for the assessment of patients with RLQ abdominal pain and possible acute appendicitis, but have NTT (Figure 2). We believe that this particular group of adult patients should be actively observed in hospital. With clinical improvement by general supportive measures and no other obvious diagnosis of concern being considered, they can be safely discharged home. However, with incomplete clinical relief, it would be prudent to further investigate those patients with US and/or CT. If results are positive for appendicitis, appendectomy should obviously be carried out, and if another pathology is diagnosed, then the patient is referred to the appropriate specialty, otherwise DL is considered and the patient is managed accordingly. If any of the markers is raised (positive triple test), the patient is further investigated and treated, as dictated by local guidelines.



**Figure 2.** Algorithm for patients with a negative triple test and suspected clinically of having acute appendicitis (C/E: clinical examination, UA: urine analysis, OPC: outpatient clinic).

From the data presented, it may be concluded that TLC, NP and CRP blood levels (triple test) should be measured upon hospital admission of adult patients with clinically suspected acute appendicitis. If used judiciously, they may spare the group of patients with NTT an unnecessary surgical operation, hence markedly reducing the NAR, preventing morbidity and minimizing the burden on hospital resources associated

with these negative explorations. Other diagnostic aids (US, CT, DL) could be reserved for only selected cases among this group of patients. Evaluation of the NTT in ruling out appendicitis among children and during pregnancy merits further investigation.

#### Conflict of interest statement

The authors do not declare any conflict of interest or financial support in this study.

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