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# BMJ Open

## Key diagnostic characteristics of fever of unknown origin in Japan: A prospective multicenter study

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4 **Key diagnostic characteristics of fever of unknown origin in Japan: A prospective**  
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7 **multicenter study**  
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13 **Running title:** Diagnosis for fever of unknown origin  
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**ABSTRACT**

**Objective:** To identify the key diagnostic features and the causes of fever of unknown origin (FUO).

**Design:** Multicenter prospective study

**Setting:** Sixteen hospitals affiliated with the Japanese Society of Hospital General Medicine, covering the East and West regions of Japan

**Participants:** Patient aged  $\geq 20$  years diagnosed with classic FUO (temperature  $\geq 38.0^{\circ}\text{C}$  at least twice within a 3-week period; cause unknown after 3 outpatient visits or 3 days of hospitalization). A total of 141 cases met the criteria and were recruited from January 2016 to December 2017.

**Intervention:** Standard diagnostic examinations

**Outcome measures:** Data collected include blood biochemical tests, inflammatory markers (erythrocyte sedimentation rate [ESR], C-reactive protein level, procalcitonin level), imaging results, autopsy findings (if performed) and final diagnosis.

**Results:** The most frequent age group was 65-79 years old and the most frequent cause of FUO was non-infectious inflammatory disease. After a 6-month follow-up period, 21.3% of cases remained undiagnosed. The types of diseases causing FUO were significantly correlated with age and prognosis. Between patients with and without a final diagnosis, the ESR differed significantly ( $p=0.041$ ).

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4 **Conclusions:** Age may be a key factor in the differential diagnosis for FUO and the  
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7 ESR test may be of value in the FUO evaluation process. These results may provide  
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10 clinicians insight into management of FUO to allow adequate treatment according to  
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13 the cause of the disease.  
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18 **Key words:** fever of unknown origin, elderly, erythrocyte sedimentation rate,  
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20 prospective studies, aging population, Japan  
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#### 23 24 25 26 27 28 **Strengths and Limitations of this study**

- 29  
30  
31 - This is the largest multicenter prospective study in Japan of fever of unknown  
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33 origin (FUO).  
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- 35  
36 - The location of the hospitals involved are scattered across the country, covering  
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38 the East and West regions of Japan, representing the largest FUO data in Japan.  
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- 41  
42 - Key diagnostic features and the causes of FUO were analyzed with respect to  
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44 patient characteristics, physical clinical findings, blood tests and imaging.  
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48 - The study included the characteristics of FUO cases whose causative disease  
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50 remained unknown after clinical investigation.  
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## INTRODUCTION

Fever of unknown origin (FUO) has many possible causes which can vary depending on region and time period.<sup>1-3</sup> FUO was first described in the medical literature in 1930.<sup>4</sup> Since then, a significantly changing spectrum of diseases causing FUO has been reported.<sup>5-10</sup> The causes of FUO have now been classified as infections, non-infectious inflammatory diseases (NIID), malignancies, other conditions and unknown.<sup>1 3</sup> The proportion of different causative disease of FUO cases has changed over time,<sup>11</sup> with fewer cases of FUO caused by infections and neoplasms over the past 40-50 years.<sup>12</sup> NIID is now the most common cause of FUO in adults,<sup>1 13</sup> while infectious diseases are most common in children.<sup>14 15</sup> In recent studies from Europe and the United States, the percentage of patients with unknown FUO varied from 7% to 53%.<sup>9</sup> Geographic factors may partly contribute to the proportion of FUO cases attributable to different causes.

Recent advances in immunohistopathology and modern imaging make the diagnosis of FUO easier, but definitive diagnosis is often difficult and cannot be achieved in up to 50% of cases.<sup>2 3 16</sup> Most previous studies of FUO have focused on its etiology and prevalence,<sup>3</sup> outcomes, or the diagnostic value of such tools as inflammatory markers<sup>17 18</sup> or positron emission tomography (PET).<sup>19-21</sup> However, limited studies have assessed the clinical utility of inflammatory markers, even though their use is now widespread.<sup>1</sup> The final diagnosis of FUO varies with age, and

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4 appearance of disease is generally nonspecific, with symptoms difficult to interpret.<sup>16</sup>

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7 <sup>22</sup> Indeed, the most difficult to diagnose cases of FUO have no signs and the causes may  
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9 remain unknown.<sup>2</sup> Thus, FUO requires a specific diagnostic approach.

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13 The medical evaluation of elderly patients requires a different perspective from  
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15 that needed for younger patients.<sup>16 23</sup> Japan has a high proportion of elderly citizens.  
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17 People aged 65 and older now constitute fully a quarter of the total population.<sup>24</sup>  
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19 Recently, the first nationwide multicenter retrospective study of FUO in Japan was  
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21 conducted, reporting the related diagnostic workup and identified diseases to consider  
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23 when evaluating FUO.<sup>1 3</sup> However, the etiology of FUO, its subjective symptoms, the  
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25 results from diagnostic tools and techniques in the elderly has not been investigated in  
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27 detail. We therefore performed this multicenter prospective study to identify the key  
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29 diagnostic features and causes of FUO with respect to patient characteristics, physical  
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31 clinical findings, blood tests and imaging. In addition, we investigated the key  
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33 characteristics of the FUO cases with no final diagnosis of the cause of FUO.  
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## 49 **PATIENTS AND METHODS**

### 50 51 **Patients**

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55 This prospective study assessed patients aged  $\geq 20$  years with classic FUO from 16  
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57 hospitals (covering the East and West regions of Japan) affiliated with the Japanese  
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4 Society of Hospital General Medicine, between January 2016 and December 2017.

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7 Classic FUO was diagnosed based on the definition used in Naito et al.<sup>1</sup> in patients  
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10 meeting all of the following criteria: 1) fever  $\geq 38.0^{\circ}\text{C}$  at least twice within a 3-week  
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13 period; 2) unknown etiology of fever after three outpatient visits or 3 days of  
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16 hospitalization; and 3) no diagnosis of immunodeficiency or confirmed human  
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19 immunodeficiency virus (HIV) infection prior to fever onset.  
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22 The following data from patients were collected during a 6-month follow-up period  
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24 and recorded on standardized case report forms: patient characteristics (sex, age,  
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26 complications, medical history); clinical findings (subjective symptoms, objective  
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28 physical findings); blood tests (blood count, general biochemical tests, inflammatory  
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30 markers: erythrocyte sedimentation rate [ESR], C-reactive protein [CRP] level,  
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32 procalcitonin level); results of blood culture if performed; results of imaging studies  
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34 and endoscopy if performed; results of cytology, histology and genetic testing or  
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Final diagnoses of the cause of FUO were classified into: infections, NIID,

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4 malignancies, other conditions and unknown. Unknown was defined as having no  
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7 definitive diagnosis after 6 months of clinical investigation.  
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## 10 **Statistical Analysis**

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13 Cross-tables were developed to present the number of patients and the percentage of  
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16 those with a final diagnosis of FUO according to subjective symptoms, examination for  
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19 diagnostic evaluation and time intervals. Chi-square test was performed to compare the  
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22 differences between different classes of final diagnosis and all listed factors. Logistic  
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25 regression models were constructed to examine the likelihood of unknown final  
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28 diagnosis. All statistic assessments were two sided and evaluated at the 0.05 level of  
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31 significance. Statistical analyses were performed using IBM SPSS Statistics for  
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34 Windows, Version 22.0. (IBM Corp., Armonk, NY, USA).  
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## 40 **RESULTS**

### 41 **Patient characteristics**

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44 A total of 141 patients who met the criteria of FUO were prospectively recruited from  
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47 16 hospitals, including 78 females (55.3%) and 63 males (44.7%), with a median age  
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50 of 62 years (range: 22–94 years; interquartile range [IQR]: 42 to 74 years). The largest  
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53 age group was those 65-79 years (n=47). Infections (n=24; 17.0%) and NIID (n=48;  
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56 34.0%) constituted the most common known causes of fever in our patient population  
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4 **(Figure 1A)**. Infectious diseases included viral infection (n=5), infectious endocarditis  
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7 (n=4) and tuberculosis (n=2). The most common NIID were Still's disease (n=7),  
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10 polymyalgia rheumatica (PMR) (n=6), antineutrophil cytoplasmic antibody-associated  
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13 vasculitis (n=6) and rheumatoid arthritis (n=4). Twenty-two patients (15.6%) were  
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16 diagnosed with malignant neoplasm, of whom 11 had malignant lymphoma. Seventeen  
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19 patients (12.1%) were diagnosed with other causes, such as histiocytic necrotizing  
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22 lymphadenitis (n=3) and subacute thyroiditis (n=2). The cause in 21.3% (n=30) of cases  
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25 remained unknown (**Table S1**). Of all FUO patients, more than 50% with infections,  
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28 malignancy, NIID and other causes required <100 days from the time of fever onset to  
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31 final diagnosis. NIID required the shortest time to be diagnosed (median 70.0 days, IQR:  
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34 54.5-107.5 days) (**Table S2**).

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37 Figure 1B and C show the distribution of the final diagnosis of FUO by sex and  
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40 age. The final diagnosis of FUO had no significant correlation with sex (**Fig 1B**;  $\chi^2=1.0$ ,  
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43  $df=4$ ,  $p=0.916$ ) but there was a significant correlation with age (**Fig 1C**;  $\chi^2=9.7$ ,  $df=4$ ,  
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46  $p=0.046$ ). NIIDs constituted the major cause among patients aged  $\geq 65$  years (43.1%)  
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49 and those <65 years (26.3 %). A lower percentage of patients aged  $\geq 65$  years (4.6%)  
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52 were diagnosed with other causative diseases compared to those aged <65 years  
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55 (18.4%).

### 56 57 58 **Symptoms and signs**

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4 The comorbidities and subjective symptoms in FUO patients by final diagnosis are  
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6 presented in **Table S3**. Comorbidities included chronic conditions such as hypertension,  
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8 diabetes and dyslipidemia. A much higher percentage of patients with comorbidities  
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10 were diagnosed with malignant neoplasm than those without (19.3% vs. 9.6%). The  
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12 major cause of FUO in patients without comorbidities was NIID (40.4%). Higher  
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14 percentages of patients with respiratory (33.3%) and gastrointestinal (23.8%)  
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16 symptoms were diagnosed with infectious diseases. Furthermore, the cause of FUO was  
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18 NIID in most patients with symptoms of arthralgia (61.4%) or muscle pain (63.2%).  
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### 28 **Biochemical and imaging results**

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30 White blood cells (WBC) and CRP were examined in all patients while 81.6% of  
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32 patients were tested for ESR and 88.7% for blood culture (**Fig S1**). Only 38.3% of  
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34 patients had the procalcitonin tests. One in four or five patients underwent imaging  
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36 scans (28.4% for Gallium Scintigraphy and 31.2% for PET). Autopsy was performed  
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38 in only 4.3% of patients. Patients who underwent an ESR test had a greater likelihood  
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40 of being diagnosed with a malignant neoplasm (17.4%) or unknown cause (25.2%)  
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42 compared to those without an ESR test. Patients who had undergone an imaging  
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44 examination had a relatively greater likelihood of being diagnosed with malignancy or  
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46 NIID compared to those without imaging examinations (Table S4).  
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58 There was a significant association between the etiology of FUO and the prognosis  
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4 of patients (**Fig 2**;  $\chi^2=27.6$ ,  $df=12$ ,  $p=0.006$ ). Most patients with FUO with different  
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7 causative diseases generally were cured or experienced relief. However, patients with  
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10 malignancy or unknown causes had higher mortality rates (22.7% and 12.9%,  
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13 respectively) (Figure 2). Among all 141 patients, the cause of fever was not identified  
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16 in 104 patients at 2 months (**Fig 3**). At the end of the follow-up period, the cause of  
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19 FUO remained unknown in 30 patients and 11 patients were not cured or had no  
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22 symptom relief. Four deaths occurred among these patients. Pathological autopsy was  
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25 performed on a small proportion of those who died ( $n=3$ ); two cases remained unknown  
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28 after autopsy (**Fig 3**).

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32 Tests were performed for diagnostic evaluation and the abnormal reading were  
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34 defined as in Naito et al.<sup>1</sup>: WBC: 4000-8000; CRP: 0.3; ESR >100 mm/h and  
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37 procalcitonin  $\geq 0.25$  ng/mL. Most patients had abnormal WBC and CRP levels (WBC:  
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40 58.1%; CRP: 74.1%, respectively) while a smaller percentage of patients had abnormal  
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43 ESR and procalcitonin levels (ESR: 23.3%; procalcitonin: 28.6%). Table 1 shows the  
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46 association of patient demographics, clinical characteristics and diagnostic  
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49 examinations for patients with known and unknown causes of FUO. There was a  
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52 significant association between having undergone ESR examination and unknown final  
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55 diagnosis of FUO (odds ratio=8.43, 95% confidence interval=1.09-65.00,  $p=0.041$ ). No  
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58 other variables differed significantly between the groups with known and unknown  
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4 cause of FUO (all  $p > 0.05$ ) (**Table 1**).  
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**Table 1.** The association of patient demographics, clinical characteristics and diagnostic evaluation between patients with known and unknown causes of FUO

Variables		Known cause <sup>a</sup>	Unknown cause <sup>a</sup>	OR (95% CI)	p-value
Age group	≥65 years	53 (81.5%)	12 (18.5%)	0.73 (0.32-1.66)	0.451
	<65 years	58 (76.3%)	18 (23.7%)	1.00	
Sex	Male	48 (76.2%)	15 (23.8%)	1.31 (0.59-2.95)	0.510
	Female	63 (80.8%)	15 (19.2%)	1.00	
Comorbidity	Yes	69 (78.4%)	19 (21.6%)	1.03 (0.44-2.37)	0.951
	No	41 (78.8%)	11 (21.2%)	1.00	
Subjective symptoms					
Headache	Yes	17 (73.9%)	6 (26.1%)	1.35 (0.48-3.80)	0.666
	No	92 (79.3%)	24 (20.7%)	1.00	
Chest pain	Yes	2 (66.7%)	1 (33.3%)	1.85 (0.16-20.07)	0.622
	No	107 (78.7%)	29 (21.3%)	1.00	
Respiratory symptoms	Yes	19 (79.2%)	5 (20.8%)	1.01 (0.34-2.98)	0.987
	No	92 (79.3%)	24 (20.7%)	1.00	
Gastrointestinal symptoms	Yes	14 (66.7%)	7 (33.3%)	2.09 (0.76-5.76)	0.155
	No	96 (80.7%)	23 (19.3%)	1.00	
Stomach ache	Yes	12 (85.7%)	2 (14.3%)	0.58 (0.12-2.73)	0.489
	No	97 (77.6%)	28 (22.4%)	1.00	
Arthralgia	Yes	38 (86.4%)	6 (13.6%)	0.47 (0.18-1.24)	0.127
	No	71 (74.7%)	24 (25.3%)	1.00	
Muscle pain	Yes	15 (78.9%)	4 (21.1%)	0.95 (0.29-3.12)	0.938

	No	93 (78.2%)	26 (21.8%)	1.00	
Lymph node swelling	Yes	13 (86.7%)	2 (13.3%)	0.53 (0.11-2.50)	0.125
	No	97 (77.6%)	28 (22.4%)	1.00	
Rash	Yes	26 (81.3%)	6 (18.8%)	0.82 (0.30-2.21)	0.92
	No	85 (78.0%)	24 (22.0%)	1.00	
Examinations					
WBC	Yes	111 (78.7%)	30 (21.3%)	NA	NA
	No	0 (0%)	0 (0%)		
CRP	Yes	111 (78.7%)	30 (21.3%)	NA	NA
	No	0 (0%)	0 (0%)		
ESR	Yes	86 (74.8%)	29 (25.2%)	8.43 (1.09-65.00)	0.041
	No	25 (96.2%)	1 (3.8%)	1.00	
Procalcitonin	Yes	41 (75.9%)	13 (24.1%)	1.31 (0.58-2.96)	0.523
	No	70 (80.5%)	17 (19.5%)	1.00	
Blood culture	Yes	96 (76.8%)	29 (23.2%)	4.53 (0.57-35.78)	0.152
	No	15 (93.8%)	1 (6.3%)	1.00	
Autopsy	Yes	4 (66.7%)	2 (33.3%)	1.88 (0.33-10.77)	0.481
	No	105 (78.9%)	28 (21.1%)	1.00	
PET	Yes	33 (75.0%)	11 (25.0%)	1.37 (0.59-3.19)	0.468
	No	78 (80.4)	19 (19.6%)	1.00	
Ga Scintigraphy	Yes	29 (72.5%)	11 (27.5%)	1.64 (0.70-3.85)	0.258
	No	82 (81.2%)	19 (18.8%)	1.00	

<sup>a</sup>Percentage was calculated as the number of patients who received an examination divided by the total patients for each condition.



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<sup>b</sup>Chi-square tests were performed.

OR: odds ratio; CI: confidence interval. NIID, non-infectious inflammatory disease; WBC, white blood cell count; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; PET, positron emission tomography; Ga, gallium.

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## DISCUSSION

This prospective multicenter study represented the largest FUO data in Japan to date. Of these 141 patients with FUO recruited from 16 hospitals, the most frequent age group was 65-79 years with the most frequent cause was NIID. There was a significant correlation between the final diagnosis of FUO and the age of patients ( $\geq 65$  and  $< 65$  years), but not with sex. While most studies have identified NIID as the most common cause of FUO in Japan,<sup>1 13 25 26</sup> our previous study in 2013 indicated that the rates of NIID as a cause of FUO were similar in participants  $\geq 65$  and  $< 65$  years.<sup>3</sup> The different selection strategies of the age groups and the aging of the Japanese population may contribute to the differences in these findings between studies. In Japan, adults aged  $\geq 65$  accounted for 26.7% of the 127.11 million population in 2016<sup>24 27</sup> and will increase to 40% in 2050, according to a new analysis.<sup>28</sup> In this study, 46.1% of patients were  $\geq 65$  years, an increase since 2013 (42.1%).<sup>3</sup> Moreover, this trend should also be considered in Western countries, where aging of the population is also expected.<sup>28</sup> A diagnosis of NIID, which occurs significantly more often in elderly patients,<sup>1</sup> must thus be considered first for FUO, particularly in patients  $\geq 65$  years.

Infections and NIID were the most common known causes of fever in our patient population. Our previous study in 2013 demonstrated that PMR and HIV should be considered as causes of FUO<sup>3</sup>. However, HIV was not found in this study, possibly due

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4 to the efficiency of HIV testing in Japan. Furthermore, the frequency of unknown cause  
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7 in our study was comparable to that found previously in 2013.<sup>3</sup>  
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10 The availability of new diagnostic techniques has changed both the spectrum of  
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12 diseases causing FUO and the time to final diagnosis. In a previous study, the cause of  
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14 FUO diagnosis  $\geq 100$  days was malignancy.<sup>3</sup> In this study, more than 50% of FUO  
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16 patients with infections, malignancy, NIID and other causes had a final diagnosis within  
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18 100 days of fever onset. Similarly, in a series of patients with FUO studied in Europe  
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20 and USA, 30-50% were of unknown cause after a follow-up of  $\geq 100$  days.<sup>6,9,29</sup>  
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28 In the present study, we evaluated key symptoms/signs in patients with FUO, to  
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30 determine which were diagnostically useful. We found that comorbidities were the  
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32 main symptoms/signs of FUO caused by malignant neoplasms. Patients with infectious  
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34 diseases often had respiratory and gastrointestinal symptoms, while those with NIID  
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36 often had arthralgia or muscle pain. Although the various symptoms/signs are not  
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38 directly related to the final diagnosis of FUO,<sup>12</sup> their presence might help improve the  
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40 differential diagnosis in patients with FUO.  
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49 A systemic review from 2003 reported that about 1.5-3% of all hospitalized patients  
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51 coped with FUO and mortality in these patients was 12-35%.<sup>30</sup> We found that the  
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53 etiology of FUO was significantly associated with prognosis and FUO patients  
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55 diagnosed with malignancy or unknown causes had higher mortality rates. A Danish  
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4 study also found that FUO patients with malignancy had poor prognosis.<sup>31</sup> Little is  
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7 known of the prognosis of FUO patients with unknown causes. In our study, 4 of 30  
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10 (13.3%) patients with unknown FUO died during within 6 months; the cause of FUO  
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13 remained unknown after autopsy in two of these patients. In patients with FUO of  
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16 unknown causes, Dutch studies showed mortality rates of 2.0-4.0%<sup>6 31</sup> and other  
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19 western-European studies reported mortality rates of 2.0-19.0%.<sup>7 10 32-34</sup> The variances  
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22 among studies may be due to differences in patient selection, study design or health  
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25 care systems.

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28 Since there is no standard diagnostic approach in FUO, classic test features are  
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31 difficult to apply in FUO studies. Of all positive biochemical tests, only 1.7%  
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34 contributed indirectly to diagnosis in a Turkey FUO study.<sup>11</sup> Despite advances in  
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37 diagnostic tests and techniques, a significant proportion of all cases remains  
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40 undiagnosed. Our study found ESR >100 mm/h in patients with FUO of unknown  
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43 causes<sup>1</sup>. In the current study, there was a significant association between unknown  
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46 cause as a final diagnosis and the performance of the ESR test but not with other  
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49 variables, such as procalcitonin or PET. In addition, only 23.3% of our patients with  
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52 unknown causes had abnormal ESR levels. Therefore, the current study allows us to  
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55 draw some conclusions on the diagnostic value of ESR; however, further investigation  
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58 is required.  
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7 In conclusion, our study identified age and ESR as potentially important factors in  
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10 the differential diagnosis for FUO. These results may allow clinicians to more quickly  
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13 determine the causes of FUO and further improve the prognosis of FUO patients.  
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## FIGURE LEGENDS

**Figure 1.** Final diagnosis of fever of unknown origin (FUO). The distribution of final diagnosis of FUO by causative disease (A), sex (B) and age group (<65 years or older) (C). Abbreviation: NIID, non-infectious inflammatory disease.

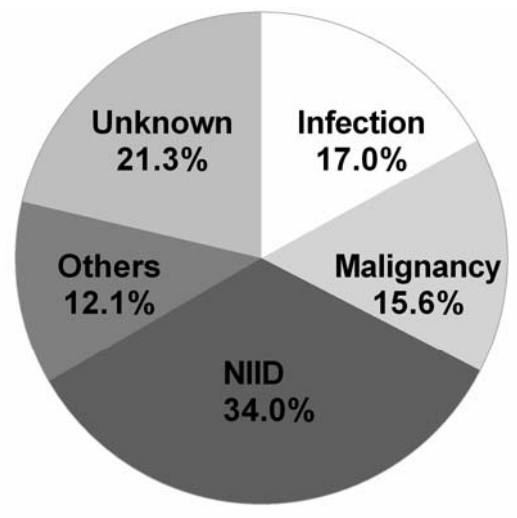
**Figure 2.** The distribution of final diagnosis of fever of unknown origin (FUO) by prognostic outcomes. There was an association between type of causative disease and prognosis ( $\chi^2=27.6$ ,  $df=12$ ,  $p=0.006$ ).

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4 **Figure 3.** Time course and prognostic outcomes for patients with fever of unknown  
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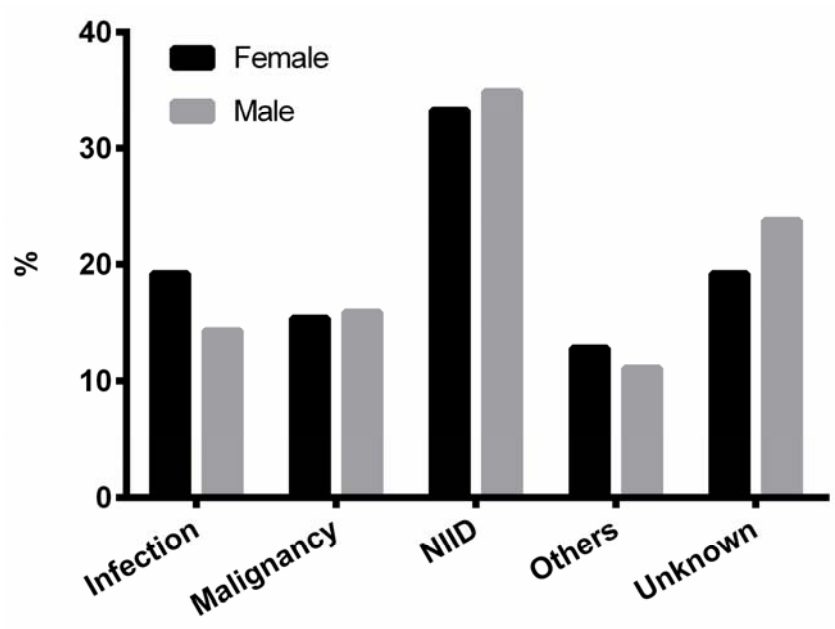
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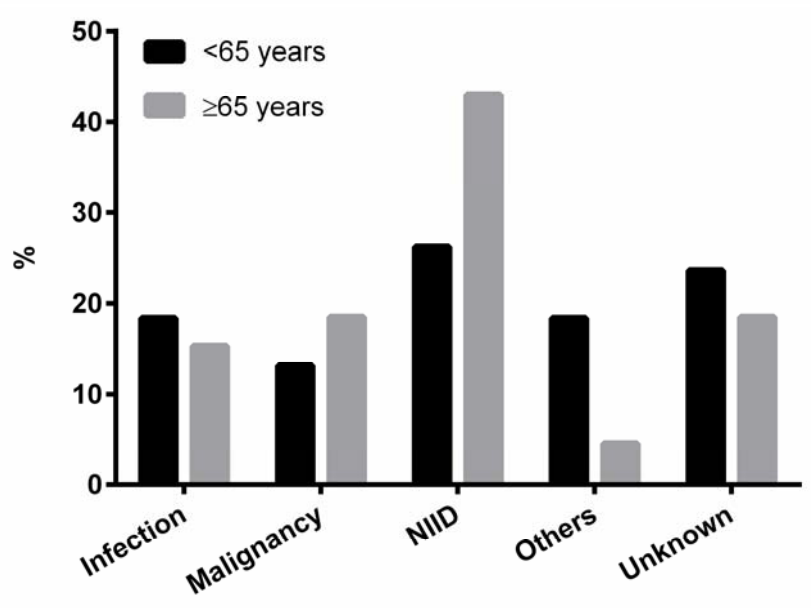


Figure 1

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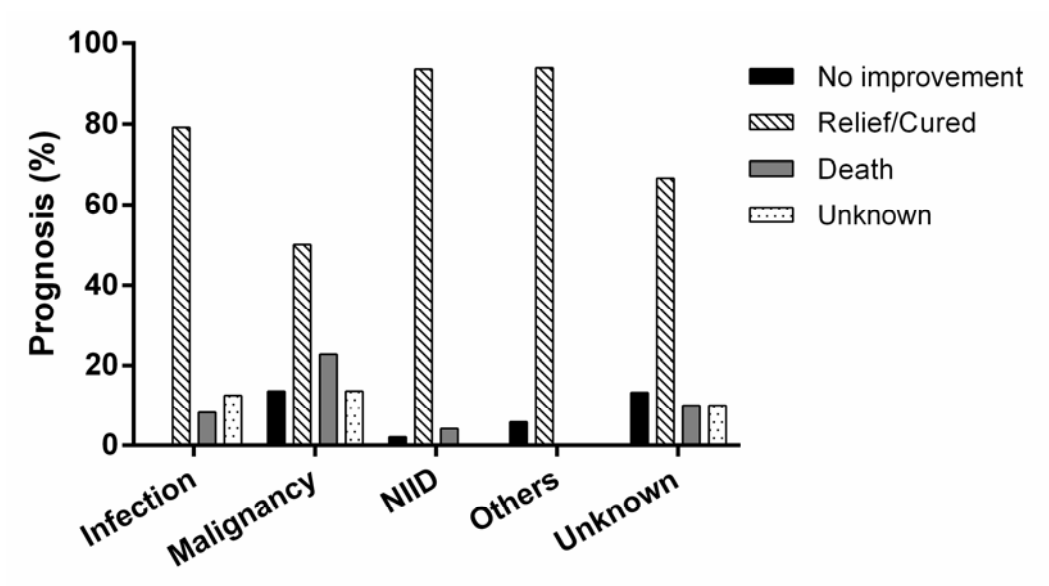


Figure 2

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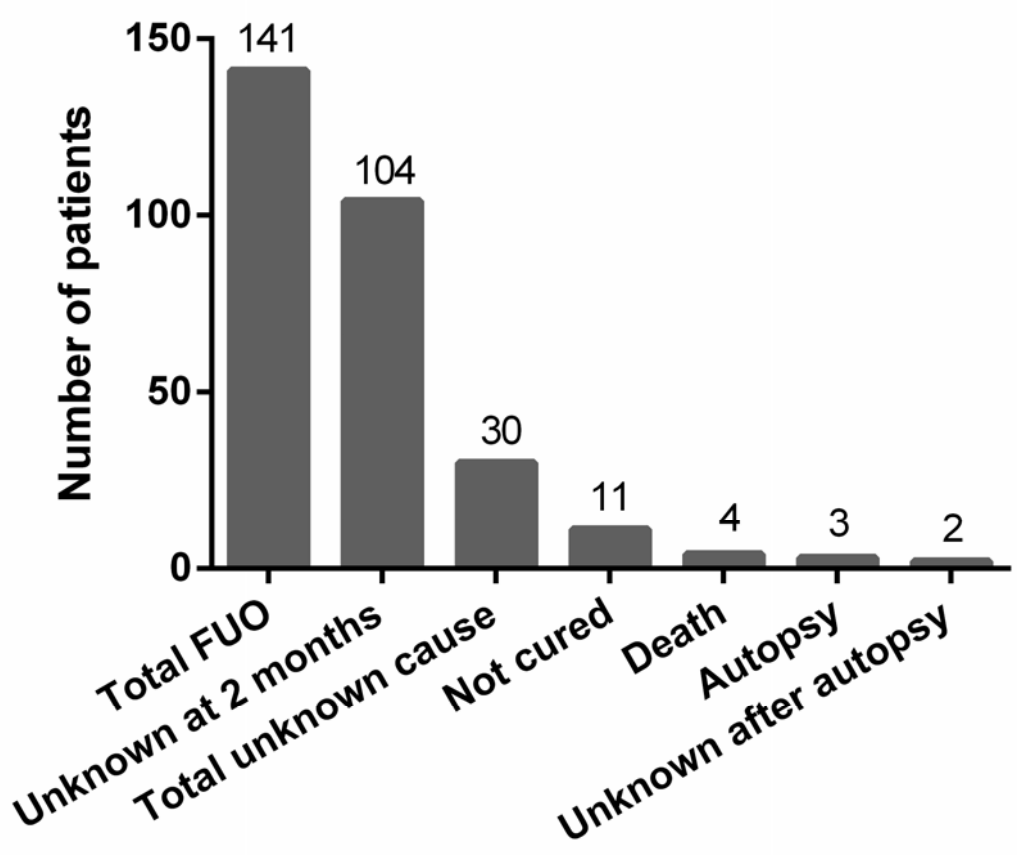
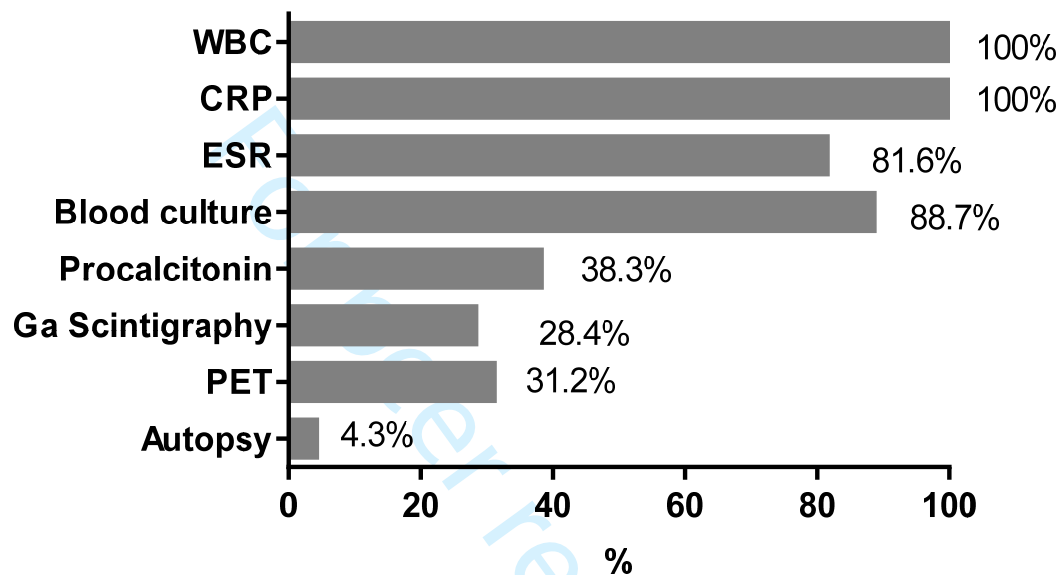


Figure 3

## Supplementary

## Supplementary Figure

## Figure S1



**Figure S1.** Frequency of examination for diagnostic evaluation. WBC, white blood cells count; CRP, C reactive protein; ESR, erythrocyte sedimentation rate; Ga, gallium; PET, positron emission tomography

## Supplementary Tables

**Table S1.** Description of final diagnosis of fever of unknown origin

Final diagnosis	n (%)
Infectious disease	24 (17.0%)
Viral infection	5
Infectious endocarditis	4
Tuberculosis	2
Malignancy	22 (15.6%)
Malignant lymphoma	11
Non-infectious inflammatory disease	48 (34.0%)
Still's disease	7
Polymyalgia rheumatica	6
ANCA-associated vasculitis	6
Rheumatoid arthritis	4
Others	17 (12.1%)
Histiocytic necrotizing lymphadenitis	3
Subacute Thyroiditis	2
Unknown	30 (21.3%)

**Table S2.** Descriptive statistics of time interval from fever onset to final diagnosis of fever of unknown origin

Final diagnosis	Time interval (days)		
	Median (IQR)	<100 days	≥100 days
Infection	70.5 (36.0, 103.5)	17 (70.8%)	7 (29.2%)
Malignancy	84.0 (54.8, 137.8)	12 (54.5%)	10 (45.5%)
NIID	70.0 (54.5, 107.5)	33 (73.3%)	12 (26.7%)
Others	75.0 (45.3, 193.8)	10 (62.5%)	6 (37.5%)

NIID, non-infectious inflammatory disease.



**Table S3.** Characteristics of patients with fever of unknown origin by types of final diagnosis

Variables <sup>a</sup>		Final diagnosis					
		Total	Infection <sup>b</sup>	Malignancy <sup>b</sup>	NIID <sup>b</sup>	Others <sup>b</sup>	Unknown <sup>b</sup>
Comorbidity	Yes	88	16 (18.2%)	17 (19.3%)	26 (29.5%)	10 (11.4%)	19 (21.6%)
	No	52	8 (15.4%)	5 (9.6%)	21 (40.4%)	7 (13.5%)	11 (21.2%)
Subjective symptoms							
Headache	Yes	23	3 (13.0%)	1 (4.3%)	9 (39.1%)	4 (17.4%)	6 (26.1%)
	No	116	20 (17.2%)	21 (18.1%)	39 (33.6%)	12 (10.3%)	24 (20.7%)
Chest pain	Yes	3	1 (33.3%)	0 (0%)	1 (33.3%)	0 (0%)	1 (33.3%)
	No	136	22 (16.2%)	22 (16.2%)	46 (33.8%)	17 (12.5%)	29 (21.3%)
Respiratory symptoms	Yes	24	8 (33.3%)	5 (20.8%)	2 (8.3%)	4 (16.7%)	5 (20.7%)
	No	116	16 (13.8%)	17 (14.7%)	46 (39.7%)	13 (11.2%)	24 (21.6%)
Gastrointestinal symptoms	Yes	21	5 (23.8%)	4 (19.0%)	3 (14.3%)	2 (9.5%)	7 (33.3%)
	No	119	19 (16.0%)	18 (15.1%)	44 (37.0%)	15 (12.6%)	23 (19.3%)
Stomach ache	Yes	14	2 (14.3%)	3 (21.4%)	5 (35.7%)	2 (14.3%)	2 (14.3%)
	No	125	21 (16.8%)	19 (15.2%)	42 (33.6%)	15 (12.0%)	28 (22.4%)
Arthralgia	Yes	44	5 (11.4%)	2 (4.5%)	27 (61.4%)	4 (9.2%)	6 (13.6%)
	No	95	18 (18.9%)	20 (21.1%)	21 (22.1%)	12 (12.6%)	24 (25.3%)
Muscle pain	Yes	19	2 (10.5%)	0 (0%)	12 (63.2%)	1 (5.3%)	4 (21.1%)
	No	119	21 (17.6%)	21 (17.6%)	36 (30.3%)	15 (12.6%)	26 (21.8%)
Lymph node swelling	Yes	15	2 (13.3%)	3 (20.0%)	3 (20.0%)	5 (33.3%)	2 (13.3%)

	No	125	21 (16.8%)	19 (15.2%)	45 (36.0%)	12 (9.6%)	28 (22.4%)
Rash	Yes	32	2 (6.3%)	6 (18.8%)	13 (40.6%)	5 (15.6%)	6 (18.8%)
	No	109	22 (20.2%)	16 (14.7%)	35 (32.1%)	12 (11.0%)	24 (22.0%)
WBC <sup>c</sup>	Yes	141	24 (17.0%)	22 (15.6%)	48 (34.0%)	17 (12.1%)	30 (21.3%)
CRP <sup>c</sup>	Yes	141	24 (15.6%)	22 (15.6%)	48 (34.0%)	17 (12.1%)	30 (21.3%)
ESR	Yes	115	14 (12.2%)	20 (17.4%)	40 (34.8%)	12 (10.4%)	29 (25.2%)
	No	26	10 (38.5%)	2 (7.7%)	8 (30.8%)	5 (19.2%)	1 (3.8%)
Procalcitonin	Yes	54	8 (14.8%)	7 (13.0%)	20 (37.0%)	6 (11.1%)	13 (24.1%)
	No	87	16 (18.4%)	15 (17.2%)	28 (32.2%)	11 (12.6%)	17 (19.5%)
Blood culture	Yes	125	23 (18.4%)	18 (14.4%)	42 (33.6%)	13 (10.4%)	29 (23.2%)
	No	16	1 (6.3%)	4 (25.0%)	6 (37.5%)	4 (25.0%)	1 (6.3%)
Autopsy	Yes	6	1 (16.7%)	1 (16.7%)	2 (33.3%)	0 (0%)	2 (33.3%)
	No	133	22 (16.5%)	20 (15.0%)	46 (34.6%)	17 (12.8%)	28 (21.1%)
PET	Yes	44	4 (9.1%)	10 (22.7%)	16 (36.4%)	3 (6.8%)	11 (25.0%)
	No	97	20 (20.6%)	12 (12.4%)	32 (33.0%)	14 (14.4%)	19 (19.6%)
Ga Scintigraphy	Yes	40	2 (5.0%)	8 (20.0%)	16 (40.0%)	3 (7.5%)	11 (27.5%)
	No	101	22 (21.8%)	14 (13.9%)	32 (31.7%)	14 (13.9%)	19 (18.8%)

NIID, non-infectious inflammatory disease. WBC, white blood cells count; CRP, C reactive protein; ESR, erythrocyte sedimentation rate; Ga, gallium; PET, positron emission tomography.

<sup>a</sup>Missing data would not be reported.

<sup>b</sup>Percentage was calculated as number of patients who performed examination divided by total patients for each condition.

<sup>c</sup>All patients performed examination of WBC and CRP.

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**Table S4.** Distribution of examinations performed for diagnostic evaluation.

Examination <sup>a</sup>		Final diagnosis					
		Total	Infection <sup>b</sup>	Malignancy <sup>b</sup>	NIID <sup>b</sup>	Others <sup>b</sup>	Unknown <sup>b</sup>
WBC <sup>c</sup>	Yes	141	24 (17.0%)	22 (15.6%)	48 (34.0%)	17 (12.1%)	30 (21.3%)
CRP <sup>c</sup>	Yes	141	24 (15.6%)	22 (15.6%)	48 (34.0%)	17 (12.1%)	30 (21.3%)
ESR	Yes	115	14 (12.2%)	20 (17.4%)	40 (34.8%)	12 (10.4%)	29 (25.2%)
	No	26	10 (38.5%)	2 (7.7%)	8 (30.8%)	5 (19.2%)	1 (3.8%)
Procalcitonin	Yes	54	8 (14.8%)	7 (13.0%)	20 (37.0%)	6 (11.1%)	13 (24.1%)
	No	87	16 (18.4%)	15 (17.2%)	28 (32.2%)	11 (12.6%)	17 (19.5%)
Blood culture	Yes	125	23 (18.4%)	18 (14.4%)	42 (33.6%)	13 (10.4%)	29 (23.2%)
	No	16	1 (6.3%)	4 (25.0%)	6 (37.5%)	4 (25.0%)	1 (6.3%)
Autopsy	Yes	6	1 (16.7%)	1 (16.7%)	2 (33.3%)	0 (0%)	2 (33.3%)
	No	133	22 (16.5%)	20 (15.0%)	46 (34.6%)	17 (12.8%)	28 (21.1%)
PET	Yes	44	4 (9.1%)	10 (22.7%)	16 (36.4%)	3 (6.8%)	11 (25.0%)
	No	97	20 (20.6%)	12 (12.4%)	32 (33.0%)	14 (14.4%)	19 (19.6%)
Ga Scintigraphy	Yes	40	2 (5.0%)	8 (20.0%)	16 (40.0%)	3 (7.5%)	11 (27.5%)
	No	101	22 (21.8%)	14 (13.9%)	32 (31.7%)	14 (13.9%)	19 (18.8%)

NIID, non-infectious inflammatory disease; WBC, white blood cells count; CRP, C reactive protein;

ESR, erythrocyte sedimentation rate; Ga, gallium; PET, positron emission tomography.

<sup>a</sup>Missing data would not be reported.

<sup>b</sup>Percentage was calculated as number of patients who performed examination divided by total patients for each condition.

<sup>c</sup>All patients performed examination of WBC and CRP

**STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology\***  
**Checklist for cohort, case-control, and cross-sectional studies (combined)**

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	3-4
<b>Introduction</b>			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5
Objectives	3	State specific objectives, including any pre-specified hypotheses	6
<b>Methods</b>			
Study design	4	Present key elements of study design early in the paper	7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7
Participants	6	(a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	7
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case	n/a
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7-8
Bias	9	Describe any efforts to address potential sources of bias	7-8
Study size	10	Explain how the study size was arrived at	n/a
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	8
		(b) Describe any methods used to examine subgroups and interactions	8
		(c) Explain how missing data were addressed	8
		(d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed	n/a

		<i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy	
		(e) Describe any sensitivity analyses	n/a
<b>Results</b>			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	n/a
		(c) Consider use of a flow diagram	n/a
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9, Table 1, figures
		(b) Indicate number of participants with missing data for each variable of interest	9
		(c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	9
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	10-11, Figures, sup tables
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	n/a
		<i>Cross-sectional study</i> —Report numbers of outcome events or summary measures	n/a
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	10-12 Figures, supp table
		(b) Report category boundaries when continuous variables were categorized	13-15
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n/a
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Supp table and supp figure
<b>Discussion</b>			
Key results	18	Summarise key results with reference to study objectives	16
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	16-18
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	16-18
Generalisability	21	Discuss the generalisability (external validity) of the study results	19
<b>Other information</b>			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	19

\*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

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2 **Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE  
3 checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at  
4 <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at [www.strobe-statement.org](http://www.strobe-statement.org).  
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# BMJ Open

## Key diagnostic characteristics of fever of unknown origin in Japanese patients: A prospective multicenter study

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2019-032059.R1
Article Type:	Original research
Date Submitted by the Author:	19-Sep-2019
Complete List of Authors:	Naito, Toshio; Juntendo University, Department of General Medicine Tanei, Mika; Juntendo University, Department of General Medicine Ikeda, Nobuhiro; Eiju General Hospital, Department of General Medicine Ishii, Toshihiro; Oita University, Department of General Medicine Suzuki, Tomio; Osaka Medical College Hospital, Department of General Medicine Morita, Hiroyuki; Gifu University Graduate School of Medicine, General Internal Medicine Yamasaki, Sho; Kyushu University Hospital, General Internal Medicine Tamura, Jun'ichi; Gunma University Graduate School of Medicine School of Medicine, General Medicine Akazawa, Kenichiro; Shonan Fujisawa Tokushukai Hospital, Internal Medicine Yamamoto, Koji; Sumitomo Hospital, General Medicine Otani, Hiroshi; Tachikawa Sogo Hospital, General Medicine Suzuki, Satoshi; Tone Chuo Byoin Kikuchi, Motoo; Nagoya City West Medical Center, Department of General medicine Ohno, Shiro; Nara Medical University, General Medicine Kobayashi, Hiroyuki; Tsukuaba University, Department of General Medicine Akita, Hozuka ; Hyogo Prefectural Kaibara Hospital, Department of Internal Medicine Tazuma, Susumu; Hiroshima University Graduate School of Biomedical & Health Sciences, Department of General Internal Medicine Hayashi, Jun; Kyushu General Internal Medicine Center, Haradoi Hospital
<b>Primary Subject Heading</b>:	Geriatric medicine
Secondary Subject Heading:	Epidemiology, Diagnostics, Geriatric medicine
Keywords:	fever of unknown origin, elderly, erythrocyte sedimentation rate, prospective studies, aging population, Japan



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7 **prospective multicenter study**  
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13 **Running title:** Diagnosis for fever of unknown origin  
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**Word Count:** 2,657

## ABSTRACT

**Objective:** To identify the key diagnostic features and causes of fever of unknown origin (FUO) in Japanese patients.

**Design:** Multicenter prospective study.

**Setting:** Sixteen hospitals affiliated with the Japanese Society of Hospital General Medicine, covering the East and West regions of Japan

**Participants:** Patient aged  $\geq 20$  years diagnosed with classic FUO (axillary temperature  $\geq 38.0^{\circ}\text{C}$  at least twice within a 3-week period, cause unknown after three outpatient visits or three days of hospitalization). A total of 141 cases met the criteria and were recruited from January 2016 to December 2017.

**Intervention:** Japanese standard diagnostic examinations

**Outcome measures:** Data collected include usual biochemical blood tests, inflammatory markers (erythrocyte sedimentation rate [ESR], C-reactive protein level, procalcitonin level), imaging results, autopsy findings (if performed) and final diagnosis.

**Results:** The most frequent age group was 65-79 years old (mean:  $58.6 \pm 9.1$  years). The most frequent cause of FUO was non-infectious inflammatory disease. After a 6-month follow-up period, 21.3% of cases remained undiagnosed. The types of diseases causing FUO were significantly correlated with age and prognosis. Between patients

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4 with and without a final diagnosis, there was no difference in CRP level between  
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7 patients with and without a final diagnosis ( $p=0.121$ ). A significant difference in  
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10 diagnosis of a causative disease was found between patients who did or did not receive  
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13 an ESR test ( $p=0.041$ ). Of the 35 patients with an abnormal ESR value, 28 (80%) had  
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16 causative disease identified.

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19 **Conclusions:** Age may be a key factor in the differential diagnosis of FUO; the ESR  
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22 test may be of value in the FUO evaluation process. These results may provide  
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25 clinicians with insight into the management of FUO to allow adequate treatment  
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28 according to the cause of the disease.

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31 **Key words:** fever of unknown origin, elderly, erythrocyte sedimentation rate,  
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34 prospective studies, aging population, Japan  
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#### 42 **Strengths and Limitations of this study**

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45 - This is the largest multicenter prospective study of fever of unknown origin  
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48 (FUO) in Japanese hospitals.
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51 - The locations of the hospitals involved are geographically dispersed across the  
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54 country, covering the eastern and western regions of Japan, representing the  
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57 largest FUO data in Japan.  
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4 - Key diagnostic features and the causes of FUO were analyzed with respect to  
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7 patients' medical histories, physical examination findings, blood tests and  
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10 imaging.  
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13 - The study included the characteristics of FUO cases whose causative disease  
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16 remained unknown after clinical investigation.  
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19 - Our study identified age and ESR test as potentially important factors useful in  
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22 assisting clinicians seeking to reveal the causes of FUO.  
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## INTRODUCTION

Fever of unknown origin (FUO) has many possible causes which can vary depending on region and time period.<sup>1-3</sup> FUO was first described in the medical literature in 1930<sup>4</sup> and defined in 1961<sup>5</sup> Since then, a significantly changing spectrum of diseases causing FUO has been reported.<sup>6-12</sup> The causes of FUO have now been classified as infections, non-infectious inflammatory diseases (NIID), malignancies, other conditions and unknown.<sup>1-3</sup> The proportion of different causative diseases of FUO has changed over time,<sup>13</sup> with fewer cases of FUO caused by infections and neoplasms over the past 40-50 years.<sup>14</sup> NIID is now the most common cause of FUO in adults,<sup>1-15</sup> while infectious diseases are most common in children.<sup>16-17</sup> In recent studies from Europe and the United States, the percentage of patients with unknown FUO varied from 7% to 53%.<sup>9</sup> Geographic factors may partly contribute to the proportion of FUO cases attributable to different causes.

Recent advances in immunohistopathology and modern imaging make the diagnosis of FUO easier, but definitive diagnosis is often difficult and cannot be achieved in up to 50% of cases.<sup>2-3 18</sup> Most previous studies of FUO have focused on its etiology and prevalence,<sup>3</sup> outcomes or the diagnostic value of such tools as inflammatory markers<sup>19-20</sup> or positron emission tomography (PET).<sup>21-24</sup> However, limited studies have assessed the clinical utility of standard inflammatory markers, even

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4 though their use is now widespread.<sup>1</sup> The final diagnosis of FUO varies with age;<sup>18 25</sup>  
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7 the most difficult to diagnose cases of FUO have no signs, with the causes remaining  
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10 unknown.<sup>2</sup> Thus, FUO requires a specific diagnostic approach.

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13 The medical evaluation of elderly patients requires a different perspective from  
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16 that needed for younger patients.<sup>18 26</sup> Japan has a high proportion of elderly citizens.  
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19 People aged 65 and older now constitute fully a quarter of the total population.<sup>27</sup>  
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22 Recently, the first nationwide multicenter retrospective study of FUO in Japan was  
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25 conducted, reporting the related diagnostic workup and identified diseases to consider  
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28 when evaluating FUO.<sup>1 3</sup> However, the etiology of FUO, its subjective symptoms and  
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31 the usefulness of diagnostic tools and techniques in diagnosing FUO in the elderly had  
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34 not been investigated in detail. The purpose of the multicenter prospective study is thus  
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37 to update the current understanding of FUO with the addition of more patients in  
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40 geographically dispersed Japanese hospitals. We aimed to identify the key symptoms  
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43 and signs, diagnostic features and causes of FUO with respect to patient medical history,  
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46 physical examination findings, standard blood tests and imaging examinations.  
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## 51 52 **PATIENTS AND METHODS**

### 53 54 55 **Patients**

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58 This prospective study assessed patients aged  $\geq 20$  years with classic FUO from 16  
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4 hospitals (encompassing the eastern and western regions of Japan) affiliated with the  
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7 Japanese Society of Hospital General Medicine, between January 2016 and December  
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10 2017. Classic FUO was diagnosed based on the definition used in Naito et al.<sup>1</sup> in  
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13 patients meeting all of the following criteria: 1) fever  $\geq 38.0^{\circ}\text{C}$  at least twice within a 3-  
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16 week period; 2) unknown etiology of fever after three outpatient visits or 3 days of  
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19 hospitalization; and 3) no diagnosis of immunodeficiency or confirmed human  
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22 immunodeficiency virus (HIV) infection prior to fever onset.  
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25 The following data from patients were collected during a 6-month follow-up period  
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28 and recorded on standardized case report forms: patient characteristics (sex, age,  
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31 comorbidities, medical history and symptoms); physical examination; blood tests  
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34 (blood count, general biochemical tests, inflammatory markers: erythrocyte  
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37 sedimentation rate [ESR], C-reactive protein [CRP] level, procalcitonin level); results  
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40 of blood culture if performed; results of imaging studies and endoscopy if performed;  
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43 results of cytology, histology and genetic testing, or autopsy findings if performed; and  
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46 final diagnosis, day of diagnosis and follow-up diagnosis outcome. In addition to  
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49 analyzing the frequency of different causative diseases and outcomes of FUO cases, we  
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52 evaluated the association between the presence or absence of examination for  
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55 diagnostic evaluation, the number of days to diagnosis and the clinical follow-up results  
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58 of inflammatory markers and other imaging tests.  
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4 Final diagnoses of the cause of FUO were classified into: infections, NIID,  
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6 malignancies, other conditions and unknown. Unknown was defined as having no  
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8 definitive diagnosis after 6 months of clinical investigation.  
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### 12 13 **Statistical Analysis**

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16 The authors developed cross-tables to present the number of patients and the percentage  
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18 of those with a final diagnosis of FUO according to symptoms, diagnostic evaluation  
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20 and time intervals. We performed Chi-square test to compare the differences between  
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22 different classes of final diagnosis and all listed factors. We constructed logistic  
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24 regression models to examine the likelihood of an unknown final diagnosis. All statistic  
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26 assessments were two sided and evaluated at the 0.05 level of significance. Statistical  
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28 analyses were performed using IBM SPSS Statistics for Windows, Version 22.0. (IBM  
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30 Corp., Armonk, NY, USA).  
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### 40 **Patient and public involvement statement**

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42 No patients or public were involved in the design and conduct of this study. Outcome  
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44 measures were not affected by patient's experience or preferences.  
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## 50 **RESULTS**

### 51 52 **Patient characteristics**

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54 A total of 141 patients who met the criteria of FUO were prospectively recruited from  
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56 16 hospitals, including 78 females (55.3%) and 63 males (44.7%), with a median age  
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4 of 62 years (range: 22–94 years; interquartile range [IQR]: 42 to 74 years). The largest  
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7 age group was those 65-79 years (n=47). Infections (n=24; 17.0%) and NIID (n=48;  
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10 34.0%) constituted the most common known causes of fever in our patient population  
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13 (**Figure 1A**). Infectious diseases included viral infection (n=5), infective endocarditis  
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16 (n=4) and tuberculosis (n=2). The most common NIID were adult-onset Still disease  
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19 (AOSD) (n=7), polymyalgia rheumatica (PMR) (n=6), antineutrophil cytoplasmic  
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22 antibody-associated vasculitis (n=6) and rheumatoid arthritis (n=4). Twenty-two  
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25 patients (15.6%) were diagnosed with malignant neoplasm, of whom 11 had malignant  
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28 lymphoma. Seventeen patients (12.1%) were diagnosed with other causes, such as  
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31 histiocytic necrotizing lymphadenitis (n=3) and subacute thyroiditis (n=2). The cause  
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34 in 21.3% (n=30) of cases remained unknown (**Table 1**). Of all FUO patients, more than  
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37 50% of those with infections, malignancy, NIID and other causes required <100 days  
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40 from the time of fever onset to the final diagnosis. NIID required the shortest time to  
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43 be diagnosed (median 70.0 days, IQR: 54.5-107.5 days) (**Table S1**).

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46 Figure 1B and C show the distribution of the final diagnosis of FUO by sex and  
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49 age. The final diagnosis of FUO had no significant correlation with sex (**Fig 1B**;  $\chi^2=1.0$ ,  
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52  $df=4$ ,  $p=0.916$ ) but there was a significant correlation with age (**Fig 1C**;  $\chi^2=9.7$ ,  $df=4$ ,  
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55  $p=0.046$ ). NIIDs constituted the major cause among patients aged  $\geq 65$  years (43.1%)  
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58 and those <65 years (26.3 %). A lower percentage of patients aged  $\geq 65$  years (4.6%)  
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4 were diagnosed with other causative diseases compared to those aged <65 years  
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7 (18.4%).  
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### 10 **Symptoms and signs**

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12 The comorbidities and symptoms in FUO patients by final diagnosis are presented in

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14 **Table 2.** Comorbidities included chronic conditions such as hypertension, diabetes and  
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16 dyslipidemia. A much higher percentage of patients with comorbidities were diagnosed  
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18 with malignant neoplasm than those without (19.3% vs. 9.6%). The major cause of  
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20 FUO in patients without comorbidities was NIID (40.4%). Higher percentages of  
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22 patients with respiratory (33.3%) and gastrointestinal (23.8%) symptoms were  
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24 diagnosed with infectious diseases. Furthermore, the cause of FUO was NIID in most  
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26 patients with symptoms of arthralgia (61.4%) or muscle pain (63.2%).  
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### 37 **Biochemical and imaging results**

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39 White blood cells (WBC) and CRP were examined in all patients, while 81.6% of  
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41 patients were tested for ESR and 88.7% for blood culture (**Fig S1**). Only 38.3% of  
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43 patients had procalcitonin tests. One in four or five patients underwent imaging scans  
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45 (28.4% for Gallium Scintigraphy and 31.2% for PET). Autopsy was performed in only  
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47 4.3% of patients. Patients who underwent an ESR test had a greater likelihood of being  
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49 diagnosed with a malignant neoplasm (17.4%) or unknown cause (25.2%) compared to  
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51 those without an ESR test. Patients who had undergone an imaging examination had a  
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4 relatively greater likelihood of being diagnosed with malignancy or NIID compared to  
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7 those without imaging examinations (**Table 2**).  
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10 There was a significant association between the etiology of FUO and the prognosis  
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12 of patients (**Fig 2**;  $\chi^2=27.6$ ,  $df=12$ ,  $p=0.006$ ). Most patients with FUO with different  
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14 causative diseases generally were cured or experienced relief. However, patients with  
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16 malignancy or unknown causes had higher mortality rates (22.7% and 12.9%,  
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18 respectively) (Figure 2). Among all 141 patients, the cause of fever was not identified  
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20 in 104 patients at 2 months (**Fig 3**). At the end of the follow-up period, the cause of  
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22 FUO remained unknown in 30 patients and 11 patients were not cured or had no  
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24 symptom relief. Four deaths occurred among these patients. Pathological autopsy was  
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26 performed on a small proportion of those who died ( $n=3$ ); two cases remained unknown  
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28 after autopsy (**Fig 3**).  
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40 Tests were performed for diagnostic evaluation and abnormal readings were defined  
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42 as in Naito et al.:<sup>1</sup> WBC: 4000-8000; CRP: 0.3; ESR >100 mm/hr and procalcitonin  
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44  $\geq 0.25$  ng/mL. Most patients with unknown cause of FUO had abnormal WBC and CRP  
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46 levels (WBC: 56.7%; CRP: 73.3%, respectively) while a smaller percentage of patients  
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48 had abnormal ESR and procalcitonin levels (ESR: 24.1%; procalcitonin: 23.1%). **Table**  
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50 **3** shows the association of patient demographics, clinical characteristics and diagnostic  
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52 examinations for patients with known and unknown causes of FUO. There was a  
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4 significant association between having undergone ESR examination and unknown final  
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7 diagnosis of FUO (odds ratio=8.43, 95% confidence interval=1.09-65.00, p=0.041).  
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10 Furthermore, 80% (28 of 35) of patients with an abnormal ESR value had a final  
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13 diagnosis. No other variables differed significantly between the groups with known and  
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16 unknown cause of FUO (all  $p>0.05$ ) (**Table 3**).  
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**Table 1.** Description of final diagnosis of fever of unknown origin

Final diagnosis	n (%)
Infectious disease	24 (17.0%)
Viral infection	5
Infective endocarditis	4
Tuberculosis	2
Malignancy	22 (15.6%)
Malignant lymphoma	11
Non-infectious inflammatory disease	48 (34.0%)
Adult-onset Still disease	7
Polymyalgia rheumatica	6
ANCA-associated vasculitis	6
Rheumatoid arthritis	4
Others	17 (12.1%)
Histiocytic necrotizing lymphadenitis	3
Subacute thyroiditis	2
Unknown	30 (21.3%)

**Table 2.** Characteristics of patients with fever of unknown origin by types of final diagnosis

Variables <sup>a</sup>		Final diagnosis					
		Total	Infection <sup>b</sup>	Malignancy <sup>b</sup>	NIID <sup>b</sup>	Other <sup>b</sup>	Unknown <sup>b</sup>
Comorbidity	Yes	88	16 (18.2%)	17 (19.3%)	26 (29.5%)	10 (11.4%)	19 (21.6%)
	No	52	8 (15.4%)	5 (9.6%)	21 (40.4%)	7 (13.5%)	11 (21.2%)
Subjective symptoms							
Headache	Yes	23	3 (13.0%)	1 (4.3%)	9 (39.1%)	4 (17.4%)	6 (26.1%)
	No	116	20 (17.2%)	21 (18.1%)	39 (33.6%)	12 (10.3%)	24 (20.7%)
Chest pain	Yes	3	1 (33.3%)	0 (0%)	1 (33.3%)	0 (0%)	1 (33.3%)
	No	136	22 (16.2%)	22 (16.2%)	46 (33.8%)	17 (12.5%)	29 (21.3%)
Respiratory symptoms	Yes	24	8 (33.3%)	5 (20.8%)	2 (8.3%)	4 (16.7%)	5 (20.7%)
	No	116	16 (13.8%)	17 (14.7%)	46 (39.7%)	13 (11.2%)	24 (21.6%)
Gastrointestinal symptoms	Yes	21	5 (23.8%)	4 (19.0%)	3 (14.3%)	2 (9.5%)	7 (33.3%)
	No	119	19 (16.0%)	18 (15.1%)	44 (37.0%)	15 (12.6%)	23 (19.3%)
Stomach ache	Yes	14	2 (14.3%)	3 (21.4%)	5 (35.7%)	2 (14.3%)	2 (14.3%)
	No	125	21 (16.8%)	19 (15.2%)	42 (33.6%)	15 (12.0%)	28 (22.4%)
Arthralgia	Yes	44	5 (11.4%)	2 (4.5%)	27 (61.4%)	4 (9.2%)	6 (13.6%)
	No	95	18 (18.9%)	20 (21.1%)	21 (22.1%)	12 (12.6%)	24 (25.3%)
Muscle pain	Yes	19	2 (10.5%)	0 (0%)	12 (63.2%)	1 (5.3%)	4 (21.1%)
	No	119	21 (17.6%)	21 (17.6%)	36 (30.3%)	15 (12.6%)	26 (21.8%)
Lymph node enlargement	Yes	15	2 (13.3%)	3 (20.0%)	3 (20.0%)	5 (33.3%)	2 (13.3%)

	No	125	21 (16.8%)	19 (15.2%)	45 (36.0%)	12 (9.6%)	28 (22.4%)
Rash	Yes	32	2 (6.3%)	6 (18.8%)	13 (40.6%)	5 (15.6%)	6 (18.8%)
	No	109	22 (20.2%)	16 (14.7%)	35 (32.1%)	12 (11.0%)	24 (22.0%)
Diagnostic Evaluation							
WBC <sup>c</sup>	Yes	141	24 (17.0%)	22 (15.6%)	48 (34.0%)	17 (12.1%)	30 (21.3%)
CRP <sup>c</sup>	Yes	141	24 (15.6%)	22 (15.6%)	48 (34.0%)	17 (12.1%)	30 (21.3%)
ESR	Yes	115	14 (12.2%)	20 (17.4%)	40 (34.8%)	12 (10.4%)	29 (25.2%)
	No	26	10 (38.5%)	2 (7.7%)	8 (30.8%)	5 (19.2%)	1 (3.8%)
Procalcitonin	Yes	54	8 (14.8%)	7 (13.0%)	20 (37.0%)	6 (11.1%)	13 (24.1%)
	No	87	16 (18.4%)	15 (17.2%)	28 (32.2%)	11 (12.6%)	17 (19.5%)
Blood culture	Yes	125	23 (18.4%)	18 (14.4%)	42 (33.6%)	13 (10.4%)	29 (23.2%)
	No	16	1 (6.3%)	4 (25.0%)	6 (37.5%)	4 (25.0%)	1 (6.3%)
Autopsy	Yes	6	1 (16.7%)	1 (16.7%)	2 (33.3%)	0 (0%)	2 (33.3%)
	No	133	22 (16.5%)	20 (15.0%)	46 (34.6%)	17 (12.8%)	28 (21.1%)
PET	Yes	44	4 (9.1%)	10 (22.7%)	16 (36.4%)	3 (6.8%)	11 (25.0%)
	No	97	20 (20.6%)	12 (12.4%)	32 (33.0%)	14 (14.4%)	19 (19.6%)
Ga Scintigraphy	Yes	40	2 (5.0%)	8 (20.0%)	16 (40.0%)	3 (7.5%)	11 (27.5%)
	No	101	22 (21.8%)	14 (13.9%)	32 (31.7%)	14 (13.9%)	19 (18.8%)

NIID, non-infectious inflammatory disease. WBC, white blood cells count; CRP, C reactive protein; ESR, erythrocyte sedimentation rate; Ga, gallium; PET, positron emission tomography.

<sup>a</sup>Missing data would not be reported.

<sup>b</sup>Percentage was calculated as number of patients who performed examination divided by total patients for each condition.

<sup>c</sup>WBC and CRP were performed on all patients



**Table 3.** The association of patient demographics, clinical characteristics and diagnostic evaluation between patients with known and unknown causes of FUO

Variables		Known cause	Unknown cause	OR (95% CI)	p-value
Age group	≥65 years	53 (81.5%)	12 (18.5%)	0.73 (0.32-1.66)	0.451
	<65 years	58 (76.3%)	18 (23.7%)	1.00	
Sex	Male	48 (76.2%)	15 (23.8%)	1.31 (0.59-2.95)	0.510
	Female	63 (80.8%)	15 (19.2%)	1.00	
Comorbidity	Yes	69 (78.4%)	19 (21.6%)	1.03 (0.44-2.37)	0.951
	No	41 (78.8%)	11 (21.2%)	1.00	
Symptoms					
Headache	Yes	17 (73.9%)	6 (26.1%)	1.35 (0.48-3.80)	0.666
	No	92 (79.3%)	24 (20.7%)	1.00	
Chest pain	Yes	2 (66.7%)	1 (33.3%)	1.85 (0.16-20.07)	0.622
	No	107 (78.7%)	29 (21.3%)	1.00	
Respiratory symptoms	Yes	19 (79.2%)	5 (20.8%)	1.01 (0.34-2.98)	0.987
	No	92 (79.3%)	24 (20.7%)	1.00	
Gastrointestinal symptoms	Yes	14 (66.7%)	7 (33.3%)	2.09 (0.76-5.76)	0.155
	No	96 (80.7%)	23 (19.3%)	1.00	
Stomach ache	Yes	12 (85.7%)	2 (14.3%)	0.58 (0.12-2.73)	0.489
	No	97 (77.6%)	28 (22.4%)	1.00	
Arthralgia	Yes	38 (86.4%)	6 (13.6%)	0.47 (0.18-1.24)	0.127
	No	71 (74.7%)	24 (25.3%)	1.00	
Muscle pain	Yes	15 (78.9%)	4 (21.1%)	0.95 (0.29-3.12)	0.938

	No	93 (78.2%)	26 (21.8%)	1.00	
Lymph node enlargement	Yes	13 (86.7%)	2 (13.3%)	0.53 (0.11-2.50)	0.125
	No	97 (77.6%)	28 (22.4%)	1.00	
Rash	Yes	26 (81.3%)	6 (18.8%)	0.82 (0.30-2.21)	0.92
	No	85 (78.0%)	24 (22.0%)	1.00	
Ancillary findings					
WBC	Yes	111 (78.7%)	30 (21.3%)	NA	NA
	No	0 (0%)	0 (0%)		
CRP	Yes	111 (78.7%)	30 (21.3%)	NA	NA
	No	0 (0%)	0 (0%)		
ESR	Yes	86 (74.8%)	29 (25.2%)	8.43 (1.09-65.00)	0.041
	No	25 (96.2%)	1 (3.8%)	1.00	
Procalcitonin	Yes	41 (75.9%)	13 (24.1%)	1.31 (0.58-2.96)	0.523
	No	70 (80.5%)	17 (19.5%)	1.00	
Blood culture	Yes	96 (76.8%)	29 (23.2%)	4.53 (0.57-35.78)	0.152
	No	15 (93.8%)	1 (6.3%)	1.00	
Autopsy	Yes	4 (66.7%)	2 (33.3%)	1.88 (0.33-10.77)	0.481
	No	105 (78.9%)	28 (21.1%)	1.00	
PET	Yes	33 (75.0%)	11 (25.0%)	1.37 (0.59-3.19)	0.468
	No	78 (80.4)	19 (19.6%)	1.00	
Ga Scintigraphy	Yes	29 (72.5%)	11 (27.5%)	1.64 (0.70-3.85)	0.258
	No	82 (81.2%)	19 (18.8%)	1.00	

<sup>a</sup>Percentage was calculated as the number of patients who received an examination divided by the total patients for each condition.

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5 <sup>b</sup>Chi-square tests were performed.

6 OR: odds ratio; CI: confidence interval; WBC, white blood cell count; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; PET, positron  
7 emission tomography; Ga, gallium.

8 Stomach ache is different from gastrointestinal symptoms, which include vomiting and diarrhea.  
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## DISCUSSION

This prospective multicenter study represents the largest report of FUO data in Japanese patients to date. Of these 141 patients with FUO recruited from 16 hospitals, the most frequent age group was 65-79 years old, with the most frequent cause being NIID.

There was a significant correlation between the final diagnosis of FUO and the age of patients ( $\geq 65$  and  $< 65$  years), but not with sex. While most studies have identified NIID as the most common cause of FUO in Japan,<sup>1 15 28 29</sup> our 2013 study found similar rates of NIID as a cause of FUO in participants  $\geq 65$  and  $< 65$  years.<sup>3</sup> The different selection strategies of the age groups and the aging of the Japanese population may contribute to the differences in these findings between studies. In Japan, adults age  $\geq 65$  accounted for 26.7% of the 127.11 million population in 2016,<sup>27 30</sup> and will increase to 40% in 2050, according to a new analysis.<sup>31</sup> In this study, 46.1% of patients were  $\geq 65$  years, an increase since 2013 (42.1%).<sup>3</sup> Moreover, this trend should also be considered in Western countries, where aging of the population is also expected.<sup>31</sup> A diagnosis of NIID, which occurs significantly more often in elderly patients,<sup>1</sup> consequently must be considered first for an FUO, particularly in patients  $\geq 65$  years. Of interest, AOSD was the most frequent NIID cause of FUO in this population. Several factors may explain this seemingly high proportion (5%). One possible justification could be that these patients may have AOSD susceptibility genes. Susceptibility of AOSD in the Japanese

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4 population depends on the genotype combinations of the HLA DRB1 and DQB1 alleles,  
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7 and predisposing risk has been found associated with the haplotype DRB1\*15:01-  
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10 DQB1\*06:02 in Japanese patients with AOSD.<sup>32</sup> However, genotyping results were not  
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13 available for this study.  
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17 Difference in causative disease between populations could be influenced by factors  
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19 such as geographic location, zoonotic characteristics and the economic and medical  
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21 organization of the local health care system. Infectious disease was the leading cause  
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23 of FUO in South-East Europe, as reported by Baymakova et al. in 2016.<sup>33</sup> Infection was  
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25 the second the most common causes of fever in our patient population. Our previous  
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27 study in 2013 demonstrated that PMR and HIV should be considered as causes of FUO.<sup>3</sup>  
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29 However, HIV was not found in this study, possibly due to the efficiency of HIV testing  
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31 in Japan. The frequency of unknown cause in our study was comparable to that found  
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33 previously in 2013.<sup>3</sup>  
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44 The availability of new diagnostic techniques, including computed tomography  
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46 (CT), PET imaging, improved culture techniques and advanced serologic assays has  
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48 changed both the spectrum of diseases causing FUO and the time to reveal the final  
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50 diagnosis. In a previous study, the cause of FUO diagnosed after  $\geq 100$  days was  
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52 malignancy.<sup>3</sup> In this study, more than 50% of FUO patients with infections, malignancy,  
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54 NIID and other causes had a final diagnosis within 100 days of fever onset. Similarly,  
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4 in a series of patients with FOU studied in Europe and USA, 30-50% were of unknown  
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7 cause after a follow-up of  $\geq 100$  days.<sup>6 9 34</sup>  
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10 In the present study, we evaluated key symptoms and signs in patients with FOU,  
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12 to determine which were diagnostically useful. We found that comorbidities were the  
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14 main symptoms and signs in FOU caused by malignant neoplasms. Patients with  
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16 infectious diseases often had respiratory and gastrointestinal symptoms, while those  
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18 with NIID often had arthralgia or muscle pain. Although the various symptoms/signs  
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20 were not directly related to the final diagnosis of FOU,<sup>14</sup> their presence might help  
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22 improve the differential diagnosis in patients with FOU.  
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31 A systemic review from 2003 reported that the prevalence of FOU was 1.5-3% in  
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33 all hospitalized patients, and mortality in these patients was 12-35%.<sup>35</sup> We found that  
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35 the etiology of FOU was significantly associated with prognosis; FOU patients  
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37 diagnosed with malignancy or unknown causes had higher mortality rates. A Danish  
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39 study also found that FOU patients with malignancy had poor prognosis.<sup>36</sup> Little is  
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41 known about the prognosis of patients with FOU of unknown cause. In our study, 4 of  
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43 30 (13.3%) patients with FOU of unknown cause died during within 6 months; the cause  
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45 of FOU remained unknown after autopsy in two of these patients. In patients with FOU  
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47 of unknown cause, Dutch studies showed mortality rates of 2.0-4.0%<sup>6 36</sup> and other  
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49 western-European studies reported mortality rates of 2.0-19.0%.<sup>7 10 37-39</sup> The variances  
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4 among studies may be due to differences in patient selection, study design or health  
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7 care systems.  
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10 Since there is no standard diagnostic approach in FUO, classic test features are  
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12 difficult to apply in FUO studies. Of all positive biochemical tests, only 1.7%  
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14 contributed indirectly to diagnosis in a Turkey FUO study.<sup>13</sup> Despite advances in  
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16 diagnostic tests and techniques, a significant proportion of all cases remains  
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18 undiagnosed.<sup>40</sup> Our previous study found that 14.9% of FUO patient had an ESR >100  
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20 mm/hr, including 5 with FUO of unknown cause<sup>1</sup>. In the current study, 35 of 115  
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22 patients (30.4%) had an abnormal ESR test result; in these, the cause of FUO was  
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24 identified in 80% of patients. In addition, there was a significant association between  
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26 known cause and ancillary ESR test, but not with other variables such as procalcitonin  
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28 or PET. Therefore, the current study demonstrated the usefulness of ESR in evaluating  
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30 FUO. However, further investigation is required. We speculate that future FUO  
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32 research may be leaving the twilight zone as diagnostic microcellular research  
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34 technologies emerge from the laboratory to point-of-care rapid diagnostic kits. We  
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36 await further advances in diagnostic artificial intelligence to expose FUO cause in more  
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38 cases.<sup>41 42</sup>  
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55 The present study has the following limitations. First, despite this being the largest  
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57 data sample ever collected from geographically-dispersed Japanese hospitals, the  
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4 sample size is still small; caution should be taken when generalizing our results. Also,  
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7 we did not establish uniformity of the diagnostic criterion used in this study, which may  
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10 have resulted in over- or under-diagnosis of specific disease categories. Uncertainty of  
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13 diagnosis was not addressed. Finally, our follow-up database was not designed to  
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16 include records of spontaneous fever remission.  
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18  
19 In conclusion, evaluating and determining the cause of a fever is complex. The  
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21 availability of new diagnostic techniques (including CT and PET imaging), improved  
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23 culture techniques and recent advances in serologic assays have all changed both the  
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25 spectrum of diseases causing FUO and the time needed to reach a final diagnosis. Our  
26  
27 study identified age and ESR as potentially important factors useful in assisting  
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29 clinicians navigate the paths to diagnosing FUO. These advances, together with future  
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31 development of multi-microbial and cancer cell detection tools, may allow faster  
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33 determination of the causes of FUO and further improve the prognosis of FUO patients.  
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58  
59 None  
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18 **Ethics approval:**

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20 Research Ethics Committee of Juntendo University School of Medicine  
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25 **Data availability statement:**

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27 All data generated within this study are available from the corresponding author on  
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29 request.  
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## FIGURE LEGENDS

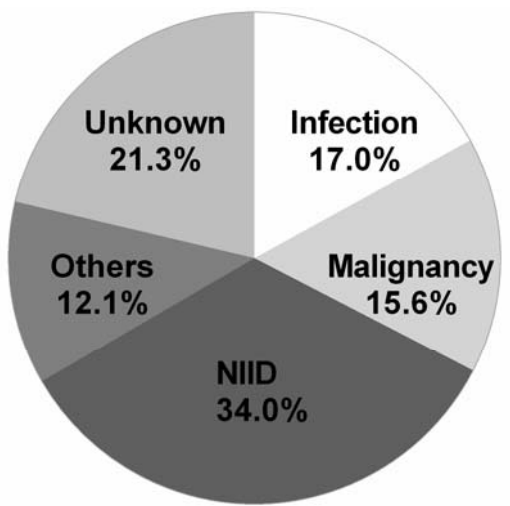
**Figure 1.** Final diagnosis of fever of unknown origin (FUO). The distribution of final diagnosis of FUO by causative disease (A), sex (B) and age group (<65 years or older) (C). Abbreviation: NIID, non-infectious inflammatory disease.

**Figure 2.** The distribution of final diagnosis of fever of unknown origin (FUO) by prognostic outcomes. There was an association between type of causative disease and prognosis ( $\chi^2=27.6$ ,  $df=12$ ,  $p=0.006$ ).

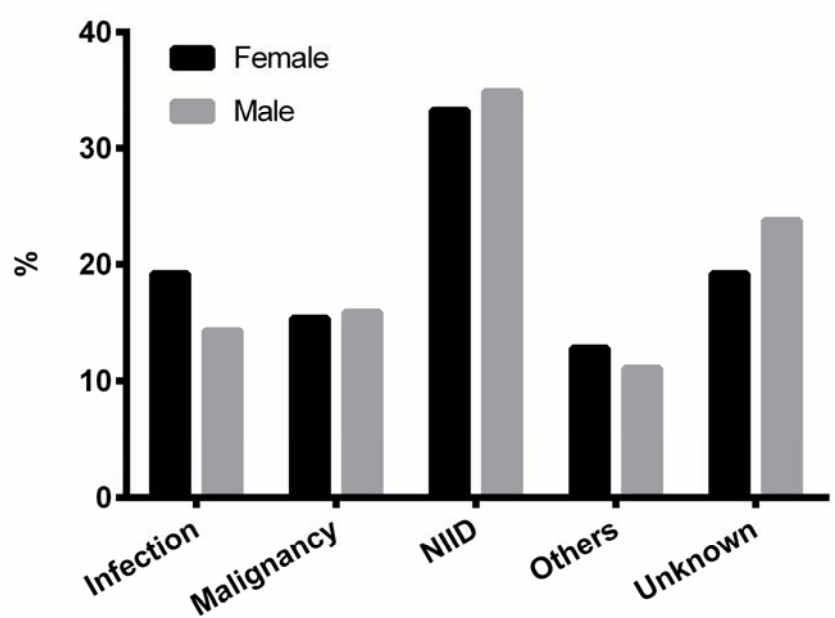
**Figure 3.** Time course and prognostic outcomes for patients with fever of unknown origin (FUO).

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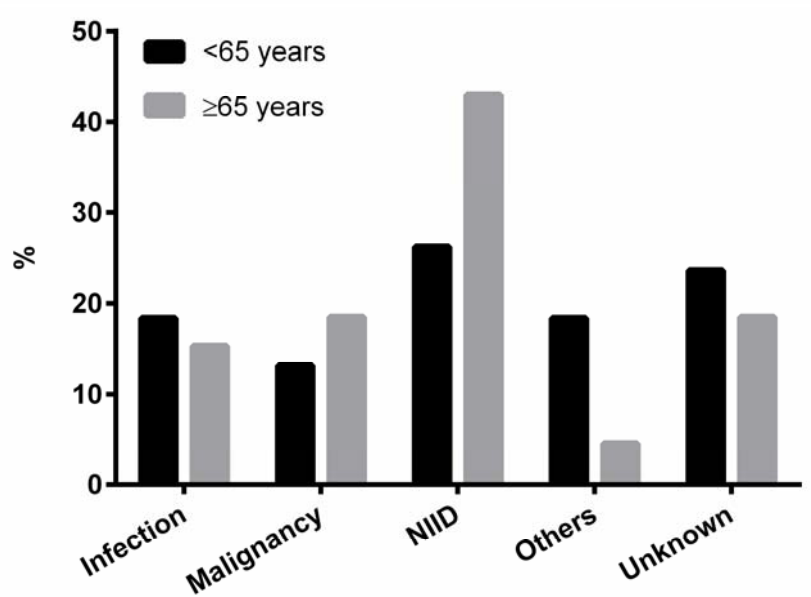


Figure 1



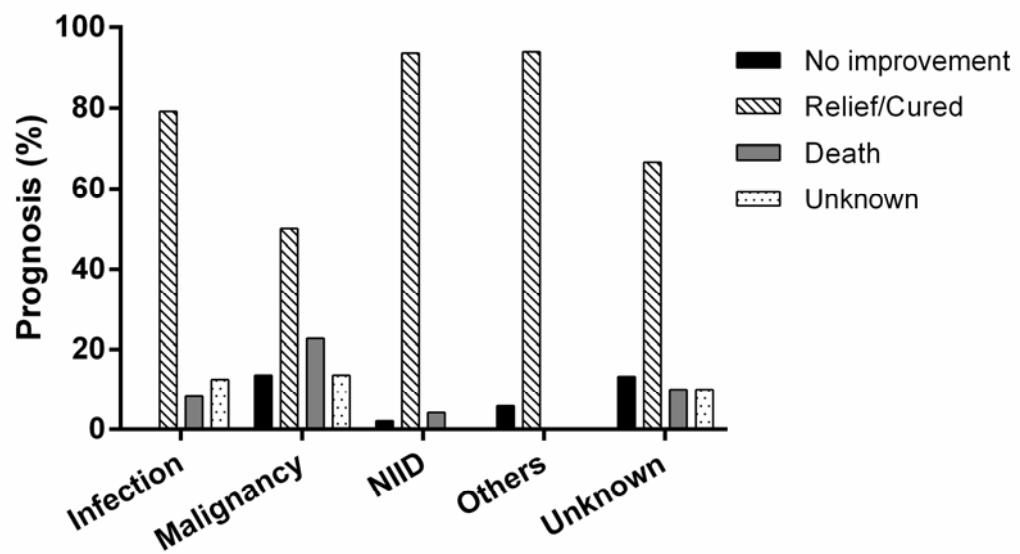


Figure 2

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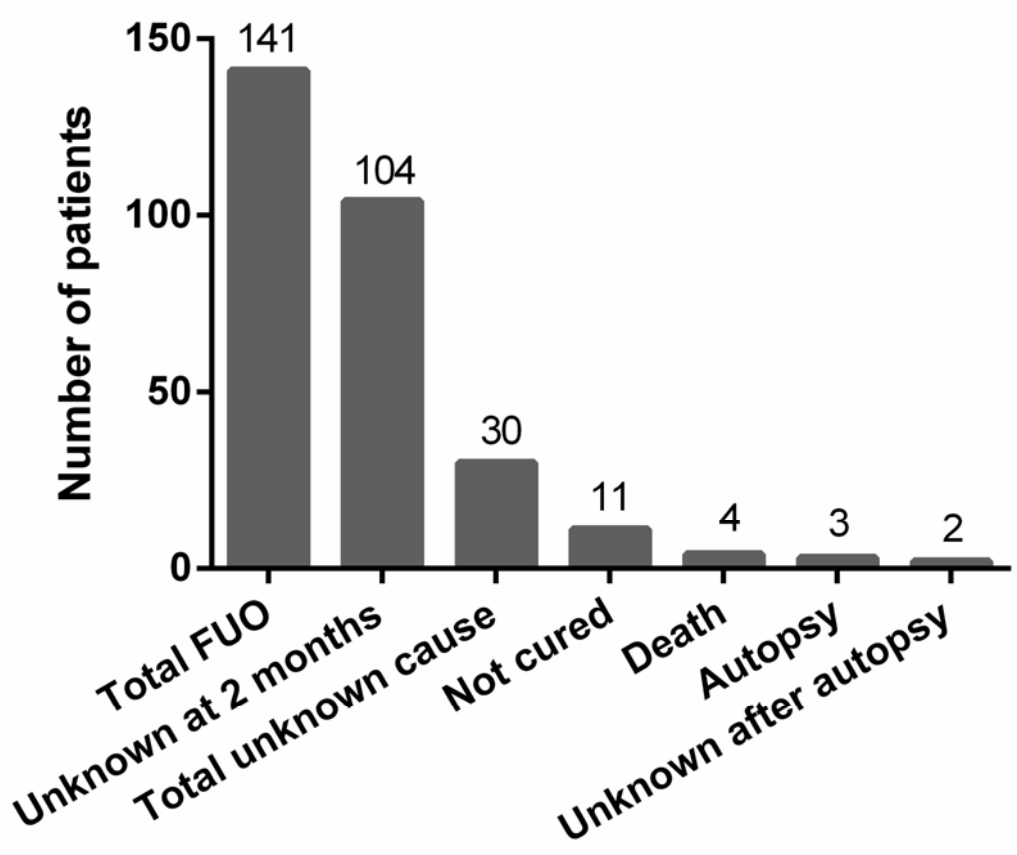
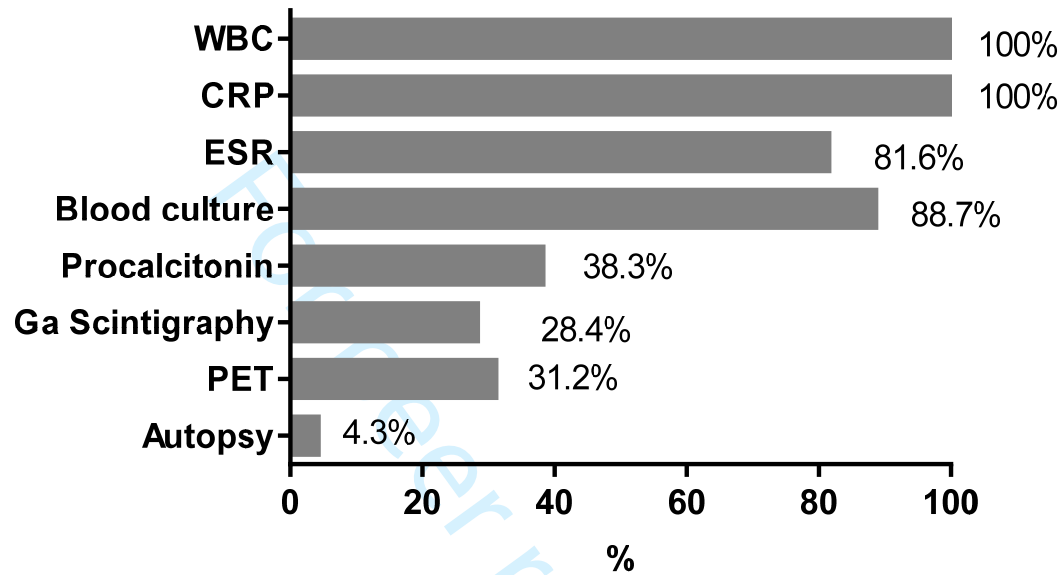


Figure 3

## Supplementary

## Supplementary Figure

## Figure S1



**Figure S1.** Frequency of examination for diagnostic evaluation. WBC, white blood cells count; CRP, C reactive protein; ESR, erythrocyte sedimentation rate; Ga, gallium; PET, positron emission tomography

## Supplementary Tables

**Table S1.** Descriptive statistics of time interval from fever onset to final diagnosis of fever of unknown origin

Final diagnosis	Time interval (days)		
	Median (IQR)	<100 days	≥100 days
Infection	70.5 (36.0, 103.5)	17 (70.8%)	7 (29.2%)
Malignancy	84.0 (54.8, 137.8)	12 (54.5%)	10 (45.5%)
NIID	70.0 (54.5, 107.5)	33 (73.3%)	12 (26.7%)
Others	75.0 (45.3, 193.8)	10 (62.5%)	6 (37.5%)

NIID, non-infectious inflammatory disease.

**STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology\***  
**Checklist for cohort, case-control, and cross-sectional studies (combined)**

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	3-4
<b>Introduction</b>			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	6
Objectives	3	State specific objectives, including any pre-specified hypotheses	7
<b>Methods</b>			
Study design	4	Present key elements of study design early in the paper	8
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	8
Participants	6	(a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	8
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case	n/a
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	8
Bias	9	Describe any efforts to address potential sources of bias	8
Study size	10	Explain how the study size was arrived at	n/a
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	8
		(b) Describe any methods used to examine subgroups and interactions	8
		(c) Explain how missing data were addressed	8
		(d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed	n/a

		<i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy	
		(e) Describe any sensitivity analyses	n/a
<b>Results</b>			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9
		(b) Give reasons for non-participation at each stage	n/a
		(c) Consider use of a flow diagram	n/a
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9, Table 1, figures
		(b) Indicate number of participants with missing data for each variable of interest	9
		(c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	9
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	10-11, Figures, sup tables
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	n/a
		<i>Cross-sectional study</i> —Report numbers of outcome events or summary measures	n/a
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	10-12 Figures, supp table
		(b) Report category boundaries when continuous variables were categorized	13-18
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n/a
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Supp table and supp figure
<b>Discussion</b>			
Key results	18	Summarise key results with reference to study objectives	16
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	19-20
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	21-22
Generalisability	21	Discuss the generalisability (external validity) of the study results	23
<b>Other information</b>			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	23

\*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

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2 **Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE  
3 checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at  
4 <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at [www.strobe-statement.org](http://www.strobe-statement.org).  
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For peer review only

# BMJ Open

## Key diagnostic characteristics of fever of unknown origin in Japanese patients: A prospective multicenter study

Journal:	<i>BMJ Open</i>
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<b>Primary Subject Heading</b>:	Geriatric medicine
Secondary Subject Heading:	Epidemiology, Diagnostics, Geriatric medicine
Keywords:	fever of unknown origin, elderly, erythrocyte sedimentation rate, prospective studies, aging population, Japan





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4 **Key diagnostic characteristics of fever of unknown origin in Japanese patients: A**  
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13 **Running title:** Diagnosis for fever of unknown origin  
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18 Toshio Naito, MD, PhD.<sup>1\*</sup>, Mika Tanei, MD, PhD.<sup>1</sup>, Nobuhiro Ikeda, MD, PhD.<sup>2</sup>,  
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**Word Count:** 2,658

## ABSTRACT

**Objective:** To identify the key diagnostic features and causes of fever of unknown origin (FUO) in Japanese patients.

**Design:** Multicenter prospective study.

**Setting:** Sixteen hospitals affiliated with the Japanese Society of Hospital General Medicine, covering the East and West regions of Japan

**Participants:** Patient aged  $\geq 20$  years diagnosed with classic FUO (axillary temperature  $\geq 38.0^{\circ}\text{C}$  at least twice within a 3-week period, cause unknown after three outpatient visits or three days of hospitalization). A total of 141 cases met the criteria and were recruited from January 2016 to December 2017.

**Intervention:** Japanese standard diagnostic examinations

**Outcome measures:** Data collected include usual biochemical blood tests, inflammatory markers (erythrocyte sedimentation rate [ESR], C-reactive protein level, procalcitonin level), imaging results, autopsy findings (if performed) and final diagnosis.

**Results:** The most frequent age group was 65-79 years old (mean:  $58.6 \pm 9.1$  years). The most frequent cause of FUO was non-infectious inflammatory disease. After a 6-month follow-up period, 21.3% of cases remained undiagnosed. The types of diseases causing FUO were significantly correlated with age and prognosis. Between patients

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4 with and without a final diagnosis, there was no difference in CRP level between  
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7 patients with and without a final diagnosis ( $p=0.121$ ). A significant difference in  
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10 diagnosis of a causative disease was found between patients who did or did not receive  
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13 an ESR test ( $p=0.041$ ). Of the 35 patients with an abnormal ESR value, 28 (80%) had  
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16 causative disease identified.

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19 **Conclusions:** Age may be a key factor in the differential diagnosis of FUO; the ESR  
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22 test may be of value in the FUO evaluation process. These results may provide  
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25 clinicians with insight into the management of FUO to allow adequate treatment  
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28 according to the cause of the disease.

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31 **Key words:** fever of unknown origin, elderly, erythrocyte sedimentation rate,  
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34 prospective studies, aging population, Japan  
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#### 42 **Strengths and Limitations of this study**

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45 - This is the largest multicenter prospective study of fever of unknown origin  
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48 (FUO) in Japanese hospitals.  
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51 - The locations of the hospitals involved are geographically dispersed across the  
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54 country, covering the eastern and western regions of Japan, representing the  
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57 largest FUO data in Japan.  
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4 - Key diagnostic features and the causes of FUO were analyzed with respect to  
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7 patients' medical histories, physical examination findings, standard blood tests  
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10 and imaging examinations.  
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13 - Despite this being the largest data sample collected from Japanese hospitals, the  
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16 sample size is still small; caution should be taken when generalizing the results.  
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## INTRODUCTION

Fever of unknown origin (FUO) has many possible causes which can vary depending on region and time period.<sup>1-3</sup> FUO was first described in the medical literature in 1930<sup>4</sup> and defined in 1961<sup>5</sup> Since then, a significantly changing spectrum of diseases causing FUO has been reported.<sup>6-12</sup> The causes of FUO have now been classified as infections, non-infectious inflammatory diseases (NIID), malignancies, other conditions and unknown.<sup>1-3</sup> The proportion of different causative diseases of FUO has changed over time,<sup>13</sup> with fewer cases of FUO caused by infections and neoplasms over the past 40-50 years.<sup>14</sup> NIID is now the most common cause of FUO in adults,<sup>1-15</sup> while infectious diseases are most common in children.<sup>16-17</sup> In recent studies from Europe and the United States, the percentage of patients with unknown FUO varied from 7% to 53%.<sup>9</sup> Geographic factors may partly contribute to the proportion of FUO cases attributable to different causes.

Recent advances in immunohistopathology and modern imaging make the diagnosis of FUO easier, but definitive diagnosis is often difficult and cannot be achieved in up to 50% of cases.<sup>2-3 18</sup> Most previous studies of FUO have focused on its etiology and prevalence,<sup>3</sup> outcomes or the diagnostic value of such tools as inflammatory markers<sup>19-20</sup> or positron emission tomography (PET).<sup>21-24</sup> However, limited studies have assessed the clinical utility of standard inflammatory markers, even

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4 though their use is now widespread.<sup>1</sup> The final diagnosis of FUO varies with age;<sup>18 25</sup>  
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7 the most difficult to diagnose cases of FUO have no signs, with the causes remaining  
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10 unknown.<sup>2</sup> Thus, FUO requires a specific diagnostic approach.

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13 The medical evaluation of elderly patients requires a different perspective from  
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16 that needed for younger patients.<sup>18 26</sup> Japan has a high proportion of elderly citizens.  
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19 People aged 65 and older now constitute fully a quarter of the total population.<sup>27</sup>  
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22 Recently, the first nationwide multicenter retrospective study of FUO in Japan was  
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25 conducted, reporting the related diagnostic workup and identified diseases to consider  
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28 when evaluating FUO.<sup>1 3</sup> However, the etiology of FUO, its subjective symptoms and  
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31 the usefulness of diagnostic tools and techniques in diagnosing FUO in the elderly had  
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34 not been investigated in detail. The purpose of the multicenter prospective study is thus  
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37 to update the current understanding of FUO with the addition of more patients in  
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40 geographically dispersed Japanese hospitals. We aimed to identify the key symptoms  
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43 and signs, diagnostic features and causes of FUO with respect to patient medical history,  
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46 physical examination findings, standard blood tests and imaging examinations.  
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## 51 **PATIENTS AND METHODS**

### 52 **Patients**

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55 This prospective study assessed patients aged  $\geq 20$  years with classic FUO from 16  
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4 hospitals (encompassing the eastern and western regions of Japan) affiliated with the  
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7 Japanese Society of Hospital General Medicine, between January 2016 and December  
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10 2017. Classic FUO was diagnosed based on the definition used in Naito et al.<sup>1</sup> in  
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13 patients meeting all of the following criteria: 1) fever  $\geq 38.0^{\circ}\text{C}$  at least twice within a 3-  
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16 week period; 2) unknown etiology of fever after three outpatient visits or 3 days of  
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19 hospitalization; and 3) no diagnosis of immunodeficiency or confirmed human  
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22 immunodeficiency virus (HIV) infection prior to fever onset.  
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25 The following data from patients were collected during a 6-month follow-up period  
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28 and recorded on standardized case report forms: patient characteristics (sex, age,  
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31 comorbidities, medical history and symptoms); physical examination; blood tests  
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34 (blood count, general biochemical tests, inflammatory markers: erythrocyte  
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37 sedimentation rate [ESR], C-reactive protein [CRP] level, procalcitonin level); results  
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40 of blood culture if performed; results of imaging studies and endoscopy if performed;  
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43 results of cytology, histology and genetic testing, or autopsy findings if performed; and  
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46 final diagnosis, day of diagnosis and follow-up diagnosis outcome. In addition to  
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49 analyzing the frequency of different causative diseases and outcomes of FUO cases, we  
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52 evaluated the association between the presence or absence of examination for  
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55 diagnostic evaluation, the number of days to diagnosis and the clinical follow-up results  
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58 of inflammatory markers and other imaging tests.  
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4 Final diagnoses of the cause of FUO were classified into: infections, NIID,  
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6 malignancies, other conditions and unknown. Unknown was defined as having no  
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8 definitive diagnosis after 6 months of clinical investigation.  
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### 12 13 **Statistical Analysis**

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16 The authors developed cross-tables to present the number of patients and the percentage  
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18 of those with a final diagnosis of FUO according to symptoms, diagnostic evaluation  
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20 and time intervals. We performed Chi-square test to compare the differences between  
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22 different classes of final diagnosis and all listed factors. We constructed logistic  
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24 regression models to examine the likelihood of an unknown final diagnosis. All statistic  
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26 assessments were two sided and evaluated at the 0.05 level of significance. Statistical  
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28 analyses were performed using IBM SPSS Statistics for Windows, Version 22.0. (IBM  
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30 Corp., Armonk, NY, USA).  
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### 40 **Patient and public involvement statement**

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42 No patients or public were involved in the design and conduct of this study. Outcome  
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44 measures were not affected by patient's experience or preferences.  
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## 50 **RESULTS**

### 51 52 **Patient characteristics**

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54 A total of 141 patients who met the criteria of FUO were prospectively recruited from  
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56 16 hospitals, including 78 females (55.3%) and 63 males (44.7%), with a median age  
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4 of 62 years (range: 22–94 years; interquartile range [IQR]: 42 to 74 years). The largest  
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7 age group was those 65-79 years (n=47). Infections (n=24; 17.0%) and NIID (n=48;  
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10 34.0%) constituted the most common known causes of fever in our patient population  
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13 (**Figure 1A**). Infectious diseases included viral infection (n=5), infective endocarditis  
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16 (n=4) and tuberculosis (n=2). The most common NIID were adult-onset Still disease  
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19 (AOSD) (n=7), polymyalgia rheumatica (PMR) (n=6), antineutrophil cytoplasmic  
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21  
22 antibody-associated vasculitis (n=6) and rheumatoid arthritis (n=4). Twenty-two  
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25 patients (15.6%) were diagnosed with malignant neoplasm, of whom 11 had malignant  
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28 lymphoma. Seventeen patients (12.1%) were diagnosed with other causes, such as  
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31 histiocytic necrotizing lymphadenitis (n=3) and subacute thyroiditis (n=2). The cause  
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33  
34 in 21.3% (n=30) of cases remained unknown (**Table 1**). Of all FUO patients, more than  
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36  
37 50% of those with infections, malignancy, NIID and other causes required <100 days  
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39  
40 from the time of fever onset to the final diagnosis. NIID required the shortest time to  
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43 be diagnosed (median 70.0 days, IQR: 54.5-107.5 days) (**Table S1**).

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45  
46 Figure 1B and C show the distribution of the final diagnosis of FUO by sex and  
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48  
49 age. The final diagnosis of FUO had no significant correlation with sex (**Fig 1B**;  $\chi^2=1.0$ ,  
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51  
52  $df=4$ ,  $p=0.916$ ) but there was a significant correlation with age (**Fig 1C**;  $\chi^2=9.7$ ,  $df=4$ ,  
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54  
55  $p=0.046$ ). NIIDs constituted the major cause among patients aged  $\geq 65$  years (43.1%)  
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58 and those <65 years (26.3 %). A lower percentage of patients aged  $\geq 65$  years (4.6%)  
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4 were diagnosed with other causative diseases compared to those aged <65 years  
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7 (18.4%).  
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### 10 **Symptoms and signs**

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12 The comorbidities and symptoms in FUO patients by final diagnosis are presented in

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14 **Table 2.** Comorbidities included chronic conditions such as hypertension, diabetes and  
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16 dyslipidemia. A much higher percentage of patients with comorbidities were diagnosed  
17  
18 with malignant neoplasm than those without (19.3% vs. 9.6%). The major cause of  
19  
20 FUO in patients without comorbidities was NIID (40.4%). Higher percentages of  
21  
22 patients with respiratory (33.3%) and gastrointestinal (23.8%) symptoms were  
23  
24 diagnosed with infectious diseases. Furthermore, the cause of FUO was NIID in most  
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26 patients with symptoms of arthralgia (61.4%) or muscle pain (63.2%).  
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### 37 **Biochemical and imaging results**

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39 White blood cells (WBC) and CRP were examined in all patients, while 81.6% of  
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41 patients were tested for ESR and 88.7% for blood culture (**Fig S1**). Only 38.3% of  
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43 patients had procalcitonin tests. One in four or five patients underwent imaging scans  
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45 (28.4% for Gallium Scintigraphy and 31.2% for PET). Autopsy was performed in only  
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47 4.3% of patients. Patients who underwent an ESR test had a greater likelihood of being  
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49 diagnosed with a malignant neoplasm (17.4%) or unknown cause (25.2%) compared to  
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51 those without an ESR test. Patients who had undergone an imaging examination had a  
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4 relatively greater likelihood of being diagnosed with malignancy or NIID compared to  
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7 those without imaging examinations (**Table 2**).  
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10 There was a significant association between the etiology of FUO and the prognosis  
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12 of patients (**Fig 2**;  $\chi^2=27.6$ ,  $df=12$ ,  $p=0.006$ ). Most patients with FUO with different  
13  
14 causative diseases generally were cured or experienced relief. However, patients with  
15  
16 malignancy or unknown causes had higher mortality rates (22.7% and 12.9%,  
17  
18 respectively) (Figure 2). Among all 141 patients, the cause of fever was not identified  
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20 in 104 patients at 2 months (**Fig 3**). At the end of the follow-up period, the cause of  
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22 FUO remained unknown in 30 patients and 11 patients were not cured or had no  
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24 symptom relief. Four deaths occurred among these patients. Pathological autopsy was  
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26 performed on a small proportion of those who died ( $n=3$ ); two cases remained unknown  
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28 after autopsy (**Fig 3**).  
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40 Tests were performed for diagnostic evaluation and abnormal readings were defined  
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42 as in Naito et al.:<sup>1</sup> WBC: 4000-8000; CRP: 0.3; ESR >100 mm/hr and procalcitonin  
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44  $\geq 0.25$  ng/mL. Most patients with unknown cause of FUO had abnormal WBC and CRP  
45  
46 levels (WBC: 56.7%; CRP: 73.3%, respectively) while a smaller percentage of patients  
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48 had abnormal ESR and procalcitonin levels (ESR: 24.1%; procalcitonin: 23.1%). **Table**  
49  
50 **3** shows the association of patient demographics, clinical characteristics and diagnostic  
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52 examinations for patients with known and unknown causes of FUO. There was a  
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4 significant association between having undergone ESR examination and unknown final  
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7 diagnosis of FUO (odds ratio=8.43, 95% confidence interval=1.09-65.00, p=0.041).  
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10 Furthermore, 80% (28 of 35) of patients with an abnormal ESR value had a final  
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13 diagnosis. No other variables differed significantly between the groups with known and  
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16 unknown cause of FUO (all p>0.05) (**Table 3**).  
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**Table 1.** Description of final diagnosis of fever of unknown origin

Final diagnosis	n (%)
Infectious disease	24 (17.0%)
Viral infection	5
Infective endocarditis	4
Tuberculosis	2
Malignancy	22 (15.6%)
Malignant lymphoma	11
Non-infectious inflammatory disease	48 (34.0%)
Adult-onset Still disease	7
Polymyalgia rheumatica	6
ANCA-associated vasculitis	6
Rheumatoid arthritis	4
Others	17 (12.1%)
Histiocytic necrotizing lymphadenitis	3
Subacute thyroiditis	2
Unknown	30 (21.3%)

**Table 2.** Characteristics of patients with fever of unknown origin by types of final diagnosis

Variables <sup>a</sup>		Final diagnosis					
		Total	Infection <sup>b</sup>	Malignancy <sup>b</sup>	NIID <sup>b</sup>	Other <sup>b</sup>	Unknown <sup>b</sup>
Comorbidity	Yes	88	16 (18.2%)	17 (19.3%)	26 (29.5%)	10 (11.4%)	19 (21.6%)
	No	52	8 (15.4%)	5 (9.6%)	21 (40.4%)	7 (13.5%)	11 (21.2%)
Subjective symptoms							
Headache	Yes	23	3 (13.0%)	1 (4.3%)	9 (39.1%)	4 (17.4%)	6 (26.1%)
	No	116	20 (17.2%)	21 (18.1%)	39 (33.6%)	12 (10.3%)	24 (20.7%)
Chest pain	Yes	3	1 (33.3%)	0 (0%)	1 (33.3%)	0 (0%)	1 (33.3%)
	No	136	22 (16.2%)	22 (16.2%)	46 (33.8%)	17 (12.5%)	29 (21.3%)
Respiratory symptoms	Yes	24	8 (33.3%)	5 (20.8%)	2 (8.3%)	4 (16.7%)	5 (20.7%)
	No	116	16 (13.8%)	17 (14.7%)	46 (39.7%)	13 (11.2%)	24 (21.6%)
Gastrointestinal symptoms	Yes	21	5 (23.8%)	4 (19.0%)	3 (14.3%)	2 (9.5%)	7 (33.3%)
	No	119	19 (16.0%)	18 (15.1%)	44 (37.0%)	15 (12.6%)	23 (19.3%)
Stomach ache	Yes	14	2 (14.3%)	3 (21.4%)	5 (35.7%)	2 (14.3%)	2 (14.3%)
	No	125	21 (16.8%)	19 (15.2%)	42 (33.6%)	15 (12.0%)	28 (22.4%)
Arthralgia	Yes	44	5 (11.4%)	2 (4.5%)	27 (61.4%)	4 (9.2%)	6 (13.6%)
	No	95	18 (18.9%)	20 (21.1%)	21 (22.1%)	12 (12.6%)	24 (25.3%)
Muscle pain	Yes	19	2 (10.5%)	0 (0%)	12 (63.2%)	1 (5.3%)	4 (21.1%)
	No	119	21 (17.6%)	21 (17.6%)	36 (30.3%)	15 (12.6%)	26 (21.8%)
Lymph node enlargement	Yes	15	2 (13.3%)	3 (20.0%)	3 (20.0%)	5 (33.3%)	2 (13.3%)



	No	125	21 (16.8%)	19 (15.2%)	45 (36.0%)	12 (9.6%)	28 (22.4%)
Rash	Yes	32	2 (6.3%)	6 (18.8%)	13 (40.6%)	5 (15.6%)	6 (18.8%)
	No	109	22 (20.2%)	16 (14.7%)	35 (32.1%)	12 (11.0%)	24 (22.0%)
Diagnostic Evaluation							
WBC <sup>c</sup>	Yes	141	24 (17.0%)	22 (15.6%)	48 (34.0%)	17 (12.1%)	30 (21.3%)
CRP <sup>c</sup>	Yes	141	24 (15.6%)	22 (15.6%)	48 (34.0%)	17 (12.1%)	30 (21.3%)
ESR	Yes	115	14 (12.2%)	20 (17.4%)	40 (34.8%)	12 (10.4%)	29 (25.2%)
	No	26	10 (38.5%)	2 (7.7%)	8 (30.8%)	5 (19.2%)	1 (3.8%)
Procalcitonin	Yes	54	8 (14.8%)	7 (13.0%)	20 (37.0%)	6 (11.1%)	13 (24.1%)
	No	87	16 (18.4%)	15 (17.2%)	28 (32.2%)	11 (12.6%)	17 (19.5%)
Blood culture	Yes	125	23 (18.4%)	18 (14.4%)	42 (33.6%)	13 (10.4%)	29 (23.2%)
	No	16	1 (6.3%)	4 (25.0%)	6 (37.5%)	4 (25.0%)	1 (6.3%)
Autopsy	Yes	6	1 (16.7%)	1 (16.7%)	2 (33.3%)	0 (0%)	2 (33.3%)
	No	133	22 (16.5%)	20 (15.0%)	46 (34.6%)	17 (12.8%)	28 (21.1%)
PET	Yes	44	4 (9.1%)	10 (22.7%)	16 (36.4%)	3 (6.8%)	11 (25.0%)
	No	97	20 (20.6%)	12 (12.4%)	32 (33.0%)	14 (14.4%)	19 (19.6%)
Ga Scintigraphy	Yes	40	2 (5.0%)	8 (20.0%)	16 (40.0%)	3 (7.5%)	11 (27.5%)
	No	101	22 (21.8%)	14 (13.9%)	32 (31.7%)	14 (13.9%)	19 (18.8%)

NIID, non-infectious inflammatory disease. WBC, white blood cells count; CRP, C reactive protein; ESR, erythrocyte sedimentation rate; Ga, gallium; PET, positron emission tomography.

<sup>a</sup>Missing data would not be reported.

<sup>b</sup>Percentage was calculated as number of patients who performed examination divided by total patients for each condition.

<sup>c</sup>WBC and CRP were performed on all patients

**Table 3.** The association of patient demographics, clinical characteristics and diagnostic evaluation between patients with known and unknown causes of FUO

Variables		Known cause	Unknown cause	OR (95% CI)	p-value
Age group	≥65 years	53 (81.5%)	12 (18.5%)	0.73 (0.32-1.66)	0.451
	<65 years	58 (76.3%)	18 (23.7%)	1.00	
Sex	Male	48 (76.2%)	15 (23.8%)	1.31 (0.59-2.95)	0.510
	Female	63 (80.8%)	15 (19.2%)	1.00	
Comorbidity	Yes	69 (78.4%)	19 (21.6%)	1.03 (0.44-2.37)	0.951
	No	41 (78.8%)	11 (21.2%)	1.00	
Symptoms					
Headache	Yes	17 (73.9%)	6 (26.1%)	1.35 (0.48-3.80)	0.666
	No	92 (79.3%)	24 (20.7%)	1.00	
Chest pain	Yes	2 (66.7%)	1 (33.3%)	1.85 (0.16-20.07)	0.622
	No	107 (78.7%)	29 (21.3%)	1.00	
Respiratory symptoms	Yes	19 (79.2%)	5 (20.8%)	1.01 (0.34-2.98)	0.987
	No	92 (79.3%)	24 (20.7%)	1.00	
Gastrointestinal symptoms	Yes	14 (66.7%)	7 (33.3%)	2.09 (0.76-5.76)	0.155
	No	96 (80.7%)	23 (19.3%)	1.00	
Stomach ache	Yes	12 (85.7%)	2 (14.3%)	0.58 (0.12-2.73)	0.489
	No	97 (77.6%)	28 (22.4%)	1.00	
Arthralgia	Yes	38 (86.4%)	6 (13.6%)	0.47 (0.18-1.24)	0.127
	No	71 (74.7%)	24 (25.3%)	1.00	
Muscle pain	Yes	15 (78.9%)	4 (21.1%)	0.95 (0.29-3.12)	0.938

	No	93 (78.2%)	26 (21.8%)	1.00	
Lymph node enlargement	Yes	13 (86.7%)	2 (13.3%)	0.53 (0.11-2.50)	0.125
	No	97 (77.6%)	28 (22.4%)	1.00	
Rash	Yes	26 (81.3%)	6 (18.8%)	0.82 (0.30-2.21)	0.92
	No	85 (78.0%)	24 (22.0%)	1.00	
Ancillary findings					
WBC	Yes	111 (78.7%)	30 (21.3%)	NA	NA
	No	0 (0%)	0 (0%)		
CRP	Yes	111 (78.7%)	30 (21.3%)	NA	NA
	No	0 (0%)	0 (0%)		
ESR	Yes	86 (74.8%)	29 (25.2%)	8.43 (1.09-65.00)	0.041
	No	25 (96.2%)	1 (3.8%)	1.00	
Procalcitonin	Yes	41 (75.9%)	13 (24.1%)	1.31 (0.58-2.96)	0.523
	No	70 (80.5%)	17 (19.5%)	1.00	
Blood culture	Yes	96 (76.8%)	29 (23.2%)	4.53 (0.57-35.78)	0.152
	No	15 (93.8%)	1 (6.3%)	1.00	
Autopsy	Yes	4 (66.7%)	2 (33.3%)	1.88 (0.33-10.77)	0.481
	No	105 (78.9%)	28 (21.1%)	1.00	
PET	Yes	33 (75.0%)	11 (25.0%)	1.37 (0.59-3.19)	0.468
	No	78 (80.4)	19 (19.6%)	1.00	
Ga Scintigraphy	Yes	29 (72.5%)	11 (27.5%)	1.64 (0.70-3.85)	0.258
	No	82 (81.2%)	19 (18.8%)	1.00	

<sup>a</sup>Percentage was calculated as the number of patients who received an examination divided by the total patients for each condition.

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5 <sup>b</sup>Chi-square tests were performed.

6 OR: odds ratio; CI: confidence interval; WBC, white blood cell count; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; PET, positron  
7 emission tomography; Ga, gallium.

8 Stomach ache is different from gastrointestinal symptoms, which include vomiting and diarrhea.  
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For peer review only

## DISCUSSION

This prospective multicenter study represents the largest report of FUO data in Japanese patients to date. Of these 141 patients with FUO recruited from 16 hospitals, the most frequent age group was 65-79 years old, with the most frequent cause being NIID.

There was a significant correlation between the final diagnosis of FUO and the age of patients ( $\geq 65$  and  $< 65$  years), but not with sex. While most studies have identified NIID as the most common cause of FUO in Japan,<sup>1 15 28 29</sup> our 2013 study found similar rates of NIID as a cause of FUO in participants  $\geq 65$  and  $< 65$  years.<sup>3</sup> The different selection strategies of the age groups and the aging of the Japanese population may contribute to the differences in these findings between studies. In Japan, adults age  $\geq 65$  accounted for 26.7% of the 127.11 million population in 2016,<sup>27 30</sup> and will increase to 40% in 2050, according to a new analysis.<sup>31</sup> In this study, 46.1% of patients were  $\geq 65$  years, an increase since 2013 (42.1%).<sup>3</sup> Moreover, this trend should also be considered in Western countries, where aging of the population is also expected.<sup>31</sup> A diagnosis of NIID, which occurs significantly more often in elderly patients,<sup>1</sup> consequently must be considered first for an FUO, particularly in patients  $\geq 65$  years. Of interest, AOSD was the most frequent NIID cause of FUO in this population. Several factors may explain this seemingly high proportion (5%). One possible justification could be that these patients may have AOSD susceptibility genes. Susceptibility of AOSD in the Japanese

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4 population depends on the genotype combinations of the HLA DRB1 and DQB1 alleles,  
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7 and predisposing risk has been found associated with the haplotype DRB1\*15:01-  
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10 DQB1\*06:02 in Japanese patients with AOSD.<sup>32</sup> However, genotyping results were not  
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13 available for this study.  
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16 Difference in causative disease between populations could be influenced by factors  
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18 such as geographic location, zoonotic characteristics and the economic and medical  
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20 organization of the local health care system. Infectious disease was the leading cause  
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22 of FUO in South-East Europe, as reported by Baymakova et al. in 2016.<sup>33</sup> Infection was  
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24 the second the most common causes of fever in our patient population. Our previous  
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26 study in 2013 demonstrated that PMR and HIV should be considered as causes of FUO.<sup>3</sup>  
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28 However, HIV was not found in this study, possibly due to the efficiency of HIV testing  
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30 in Japan. The frequency of unknown cause in our study was comparable to that found  
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32 previously in 2013.<sup>3</sup>  
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43 The availability of new diagnostic techniques, including computed tomography  
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45 (CT), PET imaging, improved culture techniques and advanced serologic assays has  
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47 changed both the spectrum of diseases causing FUO and the time to reveal the final  
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49 diagnosis. In a previous study, the cause of FUO diagnosed after  $\geq 100$  days was  
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51 malignancy.<sup>3</sup> In this study, more than 50% of FUO patients with infections, malignancy,  
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53 NIID and other causes had a final diagnosis within 100 days of fever onset. Similarly,  
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4 in a series of patients with FOU studied in Europe and USA, 30-50% were of unknown  
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7 cause after a follow-up of  $\geq 100$  days.<sup>6 9 34</sup>  
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10 In the present study, we evaluated key symptoms and signs in patients with FOU,  
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12 to determine which were diagnostically useful. We found that comorbidities were the  
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14 main symptoms and signs in FOU caused by malignant neoplasms. Patients with  
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16 infectious diseases often had respiratory and gastrointestinal symptoms, while those  
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18 with NIID often had arthralgia or muscle pain. Although the various symptoms/signs  
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20 were not directly related to the final diagnosis of FOU,<sup>14</sup> their presence might help  
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22 improve the differential diagnosis in patients with FOU.  
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31 A systemic review from 2003 reported that the prevalence of FOU was 1.5-3% in  
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33 all hospitalized patients, and mortality in these patients was 12-35%.<sup>35</sup> We found that  
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35 the etiology of FOU was significantly associated with prognosis; FOU patients  
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37 diagnosed with malignancy or unknown causes had higher mortality rates. A Danish  
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39 study also found that FOU patients with malignancy had poor prognosis.<sup>36</sup> Little is  
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41 known about the prognosis of patients with FOU of unknown cause. In our study, 4 of  
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43 30 (13.3%) patients with FOU of unknown cause died during within 6 months; the cause  
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45 of FOU remained unknown after autopsy in two of these patients. In patients with FOU  
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47 of unknown cause, Dutch studies showed mortality rates of 2.0-4.0%<sup>6 36</sup> and other  
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49 western-European studies reported mortality rates of 2.0-19.0%.<sup>7 10 37-39</sup> The variances  
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4 among studies may be due to differences in patient selection, study design or health  
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7 care systems.  
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10 Since there is no standard diagnostic approach in FUO, classic test features are  
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12 difficult to apply in FUO studies. Of all positive biochemical tests, only 1.7%  
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14 contributed indirectly to diagnosis in a Turkey FUO study.<sup>13</sup> Despite advances in  
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16 diagnostic tests and techniques, a significant proportion of all cases remains  
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18 undiagnosed.<sup>40</sup> Our previous study found that 14.9% of FUO patient had an ESR >100  
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20 mm/hr, including 5 with FUO of unknown cause<sup>1</sup>. In the current study, 35 of 115  
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22 patients (30.4%) had an abnormal ESR test result; in these, the cause of FUO was  
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24 identified in 80% of patients. In addition, there was a significant association between  
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26 known cause and ancillary ESR test, but not with other variables such as procalcitonin  
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28 or PET. Therefore, the current study demonstrated the usefulness of ESR in evaluating  
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30 FUO. However, further investigation is required. We speculate that future FUO  
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32 research may be leaving the twilight zone as diagnostic microcellular research  
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34 technologies emerge from the laboratory to point-of-care rapid diagnostic kits. We  
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36 await further advances in diagnostic artificial intelligence to expose FUO cause in more  
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38 cases.<sup>41 42</sup>  
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55 The present study has the following limitations. First, despite this being the largest  
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57 data sample ever collected from geographically-dispersed Japanese hospitals, the  
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4 sample size is still small; caution should be taken when generalizing our results. Also,  
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7 we did not establish uniformity of the diagnostic criterion used in this study, which may  
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10 have resulted in over- or under-diagnosis of specific disease categories. Uncertainty of  
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13 diagnosis was not addressed. Finally, our follow-up database was not designed to  
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16 include records of spontaneous fever remission.<sup>43</sup>  
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19 In conclusion, evaluating and determining the cause of a fever is complex. The  
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21 availability of new diagnostic techniques (including CT and PET imaging), improved  
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23 culture techniques and recent advances in serologic assays have all changed both the  
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25 spectrum of diseases causing FUO and the time needed to reach a final diagnosis. Our  
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27 study identified age and ESR as potentially important factors useful in assisting  
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29 clinicians navigate the paths to diagnosing FUO. These advances, together with future  
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31 development of multi-microbial and cancer cell detection tools, may allow faster  
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33 determination of the causes of FUO and further improve the prognosis of FUO patients.  
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#### 57 **Competing interests:**

58  
59 None  
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**Patient and public involvement statement:**

Not required. No patients or public were involved in the design and conduct of this study. Outcome measures were not affected by patient's experience or preferences.

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18 **Ethics approval:**

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20 Research Ethics Committee of Juntendo University School of Medicine  
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25 **Data availability statement:**

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27 All data generated within this study are available from the corresponding author on  
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29 request.  
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## FIGURE LEGENDS

**Figure 1.** Final diagnosis of fever of unknown origin (FUO). The distribution of final diagnosis of FUO by causative disease (A), sex (B) and age group (<65 years or older) (C). Abbreviation: NIID, non-infectious inflammatory disease.

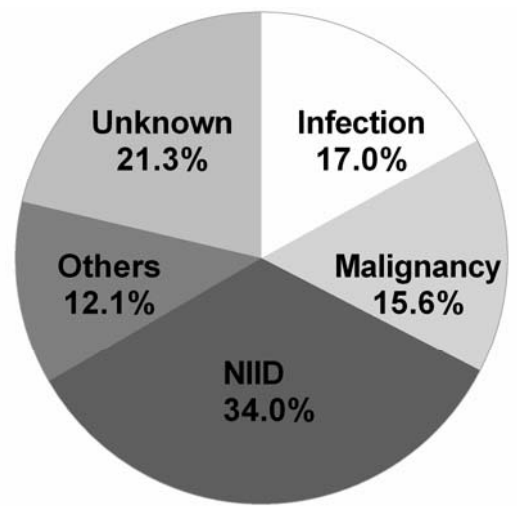
**Figure 2.** The distribution of final diagnosis of fever of unknown origin (FUO) by prognostic outcomes. There was an association between type of causative disease and prognosis ( $\chi^2=27.6$ ,  $df=12$ ,  $p=0.006$ ).

**Figure 3.** Time course and prognostic outcomes for patients with fever of unknown origin (FUO).

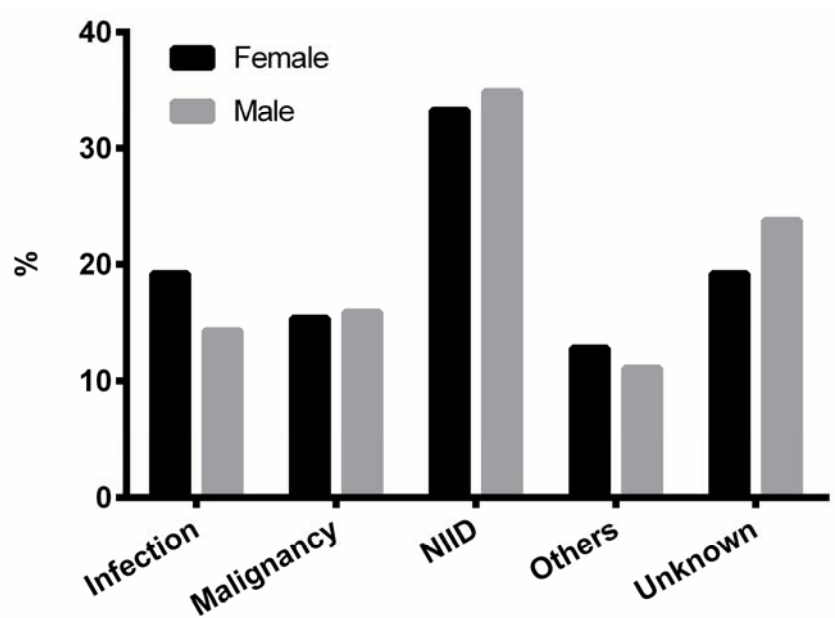


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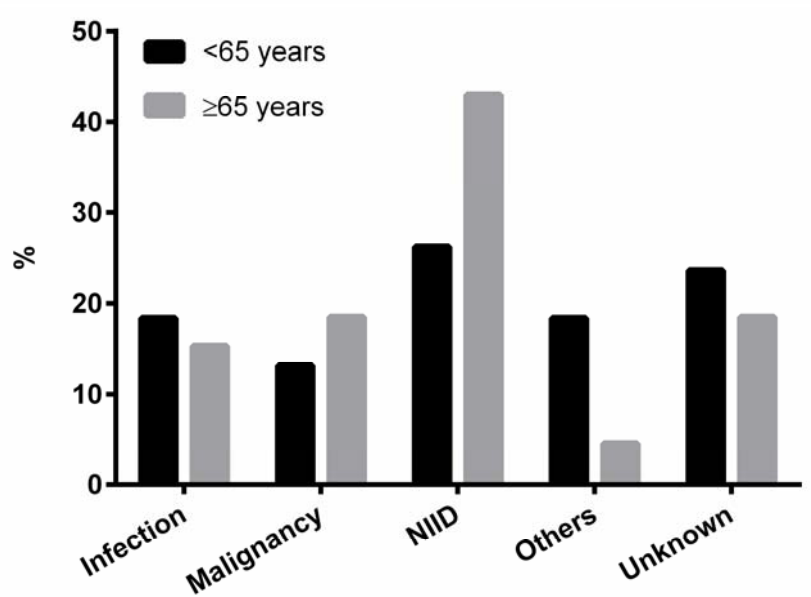


Figure 1

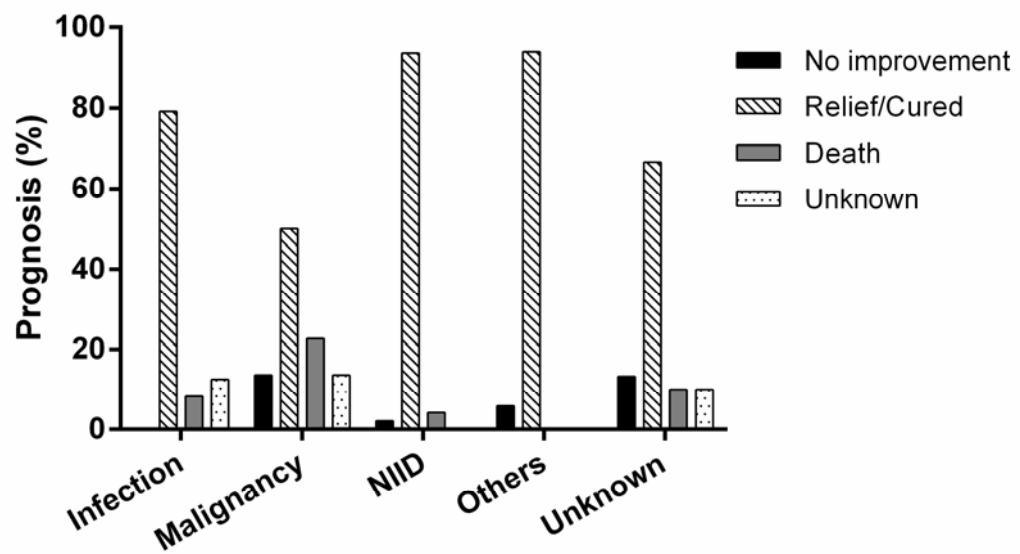


Figure 2

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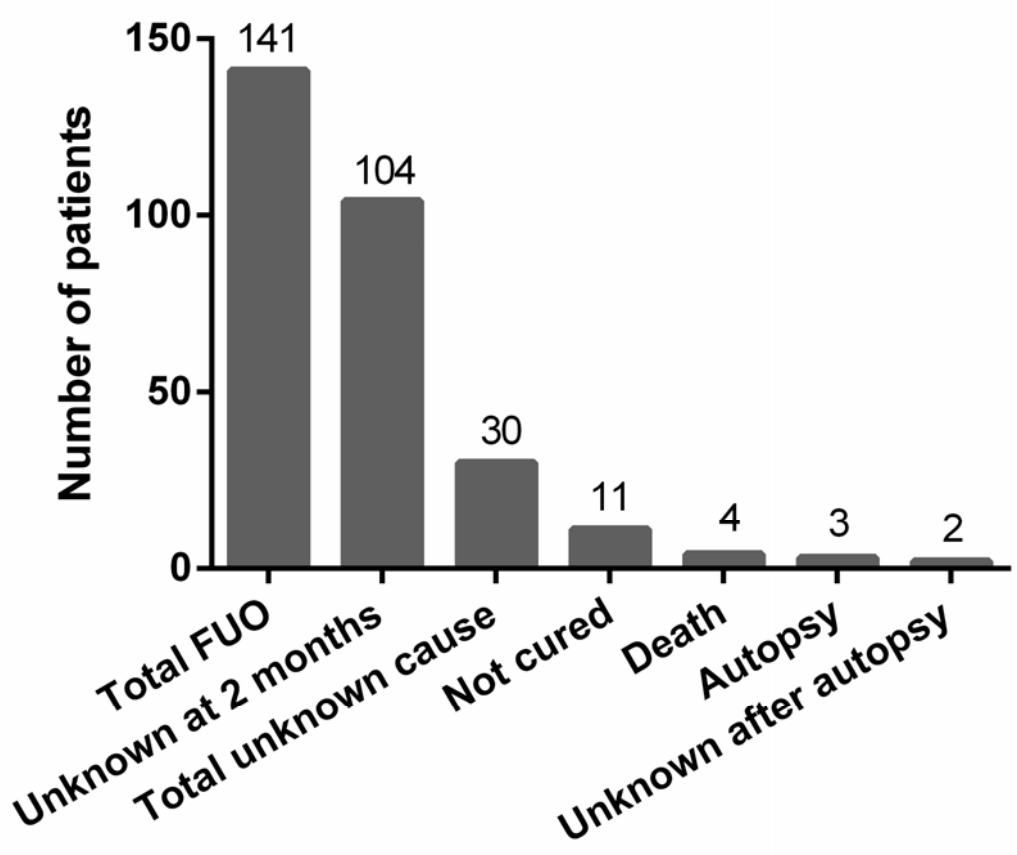
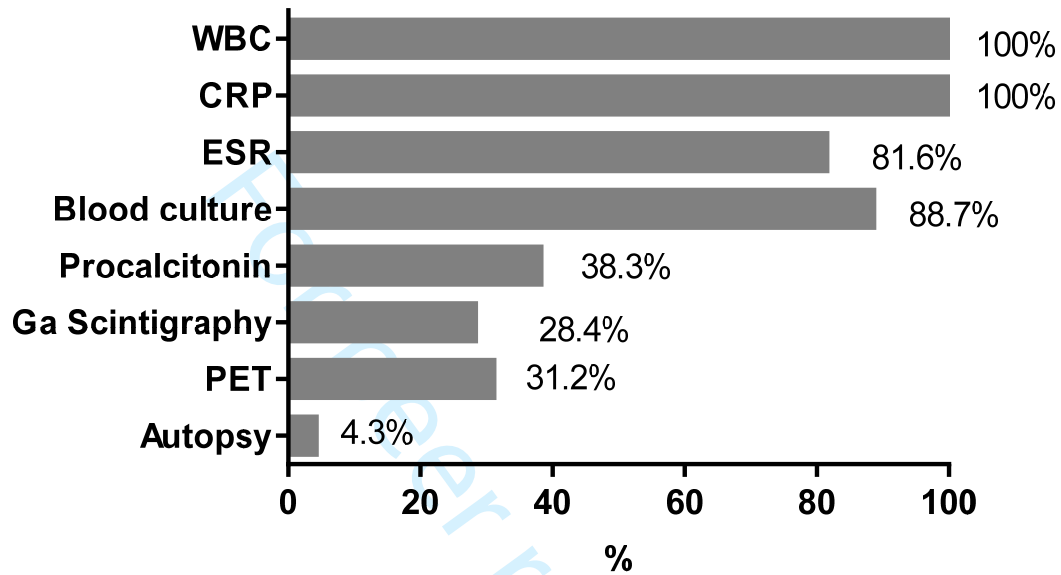


Figure 3

## Supplementary

## Supplementary Figure

## Figure S1



**Figure S1.** Frequency of examination for diagnostic evaluation. WBC, white blood cells count; CRP, C reactive protein; ESR, erythrocyte sedimentation rate; Ga, gallium; PET, positron emission tomography

## Supplementary Tables

**Table S1.** Descriptive statistics of time interval from fever onset to final diagnosis of fever of unknown origin

Final diagnosis	Time interval (days)		
	Median (IQR)	<100 days	≥100 days
Infection	70.5 (36.0, 103.5)	17 (70.8%)	7 (29.2%)
Malignancy	84.0 (54.8, 137.8)	12 (54.5%)	10 (45.5%)
NIID	70.0 (54.5, 107.5)	33 (73.3%)	12 (26.7%)
Others	75.0 (45.3, 193.8)	10 (62.5%)	6 (37.5%)

NIID, non-infectious inflammatory disease.

**STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology\***  
**Checklist for cohort, case-control, and cross-sectional studies (combined)**

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	3-4
<b>Introduction</b>			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	6
Objectives	3	State specific objectives, including any pre-specified hypotheses	7
<b>Methods</b>			
Study design	4	Present key elements of study design early in the paper	8
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	8
Participants	6	(a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	8
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case	n/a
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	8
Bias	9	Describe any efforts to address potential sources of bias	8
Study size	10	Explain how the study size was arrived at	n/a
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	8
		(b) Describe any methods used to examine subgroups and interactions	8
		(c) Explain how missing data were addressed	8
		(d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed	n/a

		<i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy	
		(e) Describe any sensitivity analyses	n/a
<b>Results</b>			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9
		(b) Give reasons for non-participation at each stage	n/a
		(c) Consider use of a flow diagram	n/a
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9, Table 1, figures
		(b) Indicate number of participants with missing data for each variable of interest	9
		(c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	9
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	10-11, Figures, sup tables
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	n/a
		<i>Cross-sectional study</i> —Report numbers of outcome events or summary measures	n/a
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	10-12 Figures, supp table
		(b) Report category boundaries when continuous variables were categorized	13-18
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n/a
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Supp table and supp figure
<b>Discussion</b>			
Key results	18	Summarise key results with reference to study objectives	16
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	19-20
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	21-22
Generalisability	21	Discuss the generalisability (external validity) of the study results	23
<b>Other information</b>			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	23

\*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

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2 **Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE  
3 checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at  
4 <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at [www.strobe-statement.org](http://www.strobe-statement.org).  
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