BMJ Open

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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-032059
Article Type:	Research
Date Submitted by the Author:	31-May-2019
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Keywords:	fever of unknown origin, elderly, erythrocyte sedimentation rate, prospective studies, aging population, Japan

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

Running title: Diagnosis for fever of unknown origin

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Word Count: 2,430

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 ≥20 years diagnosed with classic FUO (temperature ≥38.0°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).

Conclusions: Age may be a key factor in the differential diagnosis for FUO and the ESR test may be of value in the FUO evaluation process. These results may provide clinicians insight into management of FUO to allow adequate treatment according to the cause of the disease.

Key words: fever of unknown origin, elderly, erythrocyte sedimentation rate, prospective studies, aging population, Japan

Strengths and Limitations of this study

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

INTRODUCTION

Fever of unknown origin (FUO) has many possible causes which can vary depending on region and time period.[1-3] FUO was first described in the medical literature in 1930.[4] Since then, a significantly changing spectrum of diseases causing FUO has been reported.[5-10] The causes of FUO have now been classified as infections, non-infectious inflammatory diseases (NIID), malignancies, other conditions and unknown.[1 3] The proportion of different causative disease of FUO cases has changed over time,[11] with fewer cases of FUO caused by infections and neoplasms over the past 40-50 years.[12] NIID is now the most common cause of FUO in adults,[1 13] while infectious diseases are most common in children.[14 15] In recent studies from Europe and the United States, the percentage of patients with unknown FUO varied from 7% to 53%.[9] 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.[2 3 16] Most previous studies of FUO have focused on its etiology and prevalence,[3] outcomes, or the diagnostic value of such tools as inflammatory markers[17 18] or positron emission tomography (PET).[19-21] However, limited studies have assessed the clinical utility of inflammatory markers, even though their use is now widespread.[1]. The final diagnosis of FUO varies with age, and

appearance of disease is generally nonspecific, with symptoms difficult to interpret.[16 22] Indeed, the most difficult to diagnose cases of FUO have no signs and the causes may remain unknown.[2] Thus, FUO requires a specific diagnostic approach.

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

PATIENTS AND METHODS

Patients

This prospective study assessed patients aged ≥20 years with classic FUO from 16 hospitals (covering the East and West regions of Japan) affiliated with the Japanese

Society of Hospital General Medicine, between January 2016 and December 2017. Classic FUO was diagnosed based on the definition used in Naito et al.[1] in patients meeting all of the following criteria: 1) fever ≥38.0°C at least twice within a 3-week period; 2) unknown etiology of fever after three outpatient visits or 3 days of hospitalization; and 3) no diagnosis of immunodeficiency or confirmed human immunodeficiency virus (HIV) infection prior to fever onset.

The following data from patients were collected during a 6-month follow-up period and recorded on standardized case report forms: patient characteristics (sex, age, complications, medical history); clinical findings (subjective symptoms, objective physical findings); blood tests (blood count, general biochemical tests, inflammatory markers: erythrocyte sedimentation rate [ESR], C-reactive protein [CRP] level, procalcitonin level); results of blood culture if performed; results of imaging studies and endoscopy if performed; results of cytology, histology and genetic testing or autopsy findings if performed; and final diagnosis, day of diagnosis and outcome. In addition to analyzing the frequency of different causative diseases and outcomes of FUO cases, we evaluated the association between the presence or absence of examination for diagnostic evaluation, the number of days to diagnosis and the outcomes of inflammatory markers and other imaging tests.

Final diagnoses of the cause of FUO were classified into: infections, NIID,

malignancies, other conditions and unknown. Unknown was defined as having no definitive diagnosis after 6 months of clinical investigation.

Statistical Analysis

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

RESULTS

Patient characteristics

A total of 141 patients who met the criteria of FUO were prospectively recruited from 16 hospitals, including 78 females (55.3%) and 63 males (44.7%), with a median age of 62 years (range: 22–94 years; interquartile range [IQR]: 42 to 74 years). The largest age group was those 65-79 years (n=47). Infections (n=24; 17.0%) and NIID (n=48; 34.0%) constituted the most common known causes of fever in our patient population

(**Figure 1A**). Infectious diseases included viral infection (n=5), infectious endocarditis (n=4) and tuberculosis (n=2). The most common NIID were Still's disease (n=7), polymyalgia rheumatica (PMR) (n=6), antineutrophil cytoplasmic antibody-associated vasculitis (n=6) and rheumatoid arthritis (n=4). Twenty-two patients (15.6%) were diagnosed with malignant neoplasm, of whom 11 had malignant lymphoma. Seventeen patients (12.1%) were diagnosed with other causes, such as histiocytic necrotizing lymphadenitis (n=3) and subacute thyroiditis (n=2). The cause in 21.3% (n=30) of cases remained unknown (**Table S1**). Of all FUO patients, more than 50% with infections, malignancy, NIID and other causes required <100 days from the time of fever onset to final diagnosis. NIID required the shortest time to be diagnosed (median 70.0 days, IQR: 54.5-107.5 days) (**Table S2**).

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

Symptoms and signs

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

Biochemical and imaging results

White blood cells (WBC) and CRP were examined in all patients while 81.6% of patients were tested for ESR and 88.7% for blood culture **(Fig S1).** Only 38.3% of patients had the procalcitonin tests. One in four or five patients underwent imaging scans (28.4% for Gallium Scintigraphy and 31.2% for PET). Autopsy was performed in only 4.3% of patients. Patients who underwent an ESR test had a greater likelihood of being diagnosed with a malignant neoplasm (17.4%) or unknown cause (25.2%) compared to those without an ESR test. Patients who had undergone an imaging examination had a relatively greater likelihood of being diagnosed with malignancy or NIID compared to those without imaging examinations (Table S4).

There was a significant association between the etiology of FUO and the prognosis

of patients (**Fig 2**; $\chi 2=27.6$, df=12, p=0.006). Most patients with FUO with different causative diseases generally were cured or experienced relief. However, patients with malignancy or unknown causes had higher mortality rates (22.7% and 12.9%, respectively) (Figure 2). Among all 141 patients, the cause of fever was not identified in 104 patients at 2 months (**Fig 3**). At the end of the follow-up period, the cause of FUO remained unknown in 30 patients and 11 patients were not cured or had no symptom relief. Four deaths occurred among these patients. Pathological autopsy was performed on a small proportion of those who died (n=3); two cases remained unknown after autopsy (**Fig 3**).

Tests were performed for diagnostic evaluation and the abnormal reading were defined as in Naito et al.[1]: WBC: 4000-8000; CRP: 0.3; ESR >100 mm/h and procalcitonin ≥0.25 ng/mL. Most patients had abnormal WBC and CRP levels (WBC: 58.1%; CRP: 74.1%, respectively) while a smaller percentage of patients had abnormal ESR and procalcitonin levels (ESR: 23.3%; procalcitonin: 28.6%). Table 1 shows the association of patient demographics, clinical characteristics and diagnostic examinations for patients with known and unknown causes of FUO. There was a significant association between having undergone ESR examination and unknown final diagnosis of FUO (odds ratio=8.43, 95% confidence interval=1.09-65.00, p=0.041). No other variables differed significantly between the groups with known and unknown

cause of FUO (all p>0.05) (**Table 1**).

Table 1. The association of patient demographics, clinical characteristics and diagnostic evaluation between patients with known and unknown causes of FUO

Variables		Known cause[a]	Unknown cause[a]	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)	0.566
	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)	0.255
	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)	0.425
	No	97 (77.6%)	28 (22.4%)	1.00	
Rash	Yes	26 (81.3%)	6 (18.8%)	0.82 (0.30-2.21)	0.692
	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	

[a]Percentage was calculated as the number of patients who received an examination divided by the total patients for each condition.

[b]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.

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 (≥65 and <65 years), but not with sex. While most studies have identified NIID as the most common cause of FUO in Japan,[1 13 25 26] our previous study in 2013 indicated that the rates of NIID as a cause of FUO were similar in participants ≥65 and <65 years.[3] 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 ≥65 accounted for 26.7% of the 127.11 million population in 2016[24 27] and will increase to 40% in 2050, according to a new analysis.[28] In this study, 46.1% of patients were ≥65 years, an increase since 2013 (42.1%).[3] Moreover, this trend should also be considered in Western countries, where aging of the population is also expected.[28] A diagnosis of NIID, which occurs significantly more often in elderly patients,[1] must thus be considered first for FUO, particularly in patients ≥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[3]. However, HIV was not found in this study, possibly due

to the efficiency of HIV testing in Japan. Furthermore, the frequency of unknown cause in our study was comparable to that found previously in 2013.[3]

The availability of new diagnostic techniques has changed both the spectrum of diseases causing FUO and the time to final diagnosis. In a previous study, the cause of FUO diagnosis ≥100 days was malignancy.[3] In this study, more than 50% of FUO patients with infections, malignancy, NIID and other causes had a final diagnosis within 100 days of fever onset. Similarly, in a series of patients with FUO studied in Europe and USA, 30-50% were of unknown cause after a follow-up of ≥100 days.[6 9 29]

In the present study, we evaluated key symptoms/signs in patients with FUO, to determine which were diagnostically useful. We found that comorbidities were the main symptoms/signs of FUO caused by malignant neoplasms. Patients with infectious diseases often had respiratory and gastrointestinal symptoms, while those with NIID often had arthralgia or muscle pain. Although the various symptoms/signs are not directly related to the final diagnosis of FUO,[12] their presence might help improve the differential diagnosis in patients with FUO.

A systemic review from 2003 reported that about 1.5-3% of all hospitalized patients coped with FUO and mortality in these patients was 12-35%.[30] We found that the etiology of FUO was significantly associated with prognosis and FUO patients diagnosed with malignancy or unknown causes had higher mortality rates. A Danish

study also found that FUO patients with malignancy had poor prognosis.[31] Little is known of the prognosis of FUO patients with unknown causes. In our study, 4 of 30 (13.3%) patients with unknown FUO died during within 6 months; the cause of FUO remained unknown after autopsy in two of these patients. In patients with FUO of unknown causes, Dutch studies showed mortality rates of 2.0-4.0%[6 31] and other western-European studies reported mortality rates of 2.0-19.0%.[7 10 32-34] The variances among studies may be due to differences in patient selection, study design or health care systems.

Since there is no standard diagnostic approach in FUO, classic test features are difficult to apply in FUO studies. Of all positive biochemical tests, only 1.7% contributed indirectly to diagnosis in a Turkey FUO study.[11] Despite advances in diagnostic tests and techniques, a significant proportion of all cases remains undiagnosed. Our study found ESR >100 mm/h in patients with FUO of unknown causes[1]. In the current study, there was a significant association between unknown cause as a final diagnosis and the performance of the ESR test but not with other variables, such as procalcitonin or PET. In addition, only 23.3% of our patients with unknown causes had abnormal ESR levels. Therefore, the current study allows us to draw some conclusions on the diagnostic value of ESR; however, further investigation is required.

In conclusion, our study identified age and ESR as potentially important factors in the differential diagnosis for FUO. These results may allow clinicians to more quickly determine the causes of FUO and further improve the prognosis of FUO patients.

Funding: This work was supported by Grants-in-Aid for Scientific Research, Japan Project Number: 16K09257.

Competing interests: None

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Acknowledgments

We would like to acknowledge Convergence CT Japan K.K for conducting statistical analysis, interpretation of data, and reviewing this manuscript. We would also like to acknowledge support from Tatsuo Ishizuka, Kenji Kanazawa, Nobuki Nanki, Megumi Higaki, and Koichi Mashiba for valuable administrative and research assistance throughout this study.

Ethics approval:

Research Ethics Committee of Juntendo University School of Medicine

Data sharing statement: No additional data are available.

REFERENCES

1. Naito T, Torikai K, Mizooka M, et al. Relationships between Causes of Fever of Unknown Origin and Inflammatory Markers: A Multicenter Collaborative Retrospective Study. *Intern Med* 2015;54(16):1989-94. doi: 10.2169/internalmedicine.54.3313 [published Online First: 2015/08/19]
2. Cunha BA, Lortholary O, Cunha CB. Fever of unknown origin: a clinical approach. *Am J Med* 2015;128(10):1138 e1-38 e15. doi: 10.1016/j.amjmed.2015.06.001 [published Online First: 2015/06/21]
3. Naito T, Mizooka M, Mitsumoto F, et al. Diagnostic workup for fever of unknown origin: a multicenter collaborative retrospective study. *BMJ Open* 2013;3(12):e003971. doi: 10.1136/bmjopen-2013-003971 [published Online First: 2013/12/24]
4. Alt HL, Barker MH. Fever of unknown origin. *JAMA* 1930;94:1457–61.
5. Petersdorf RG, Beeson PB. Fever of unexplained origin: report on 100 cases. *Medicine (Baltimore)* 1961;40:1-30. [published Online First: 1961/02/01]
6. de Kleijn EM, Vandenbroucke JP, van der Meer JW. Fever of unknown origin (FUO). I A. prospective multicenter study of 167 patients with FUO, using fixed epidemiologic entry criteria. The Netherlands FUO Study Group. *Medicine (Baltimore)* 1997;76(6):392-400. [published Online First: 1997/12/31]
7. Zenone T. Fever of unknown origin in adults: evaluation of 144 cases in a non-university hospital. *Scand J Infect Dis* 2006;38(8):632-8. doi:

10.1080/00365540600606564 [published Online First: 2006/07/22]

8. Miller RF, Hingorami AD, Foley NM. Pyrexia of undetermined origin in patients with human immunodeficiency virus infection and AIDS. *Int J STD AIDS* 1996;7(3):170-5. doi: 10.1258/0956462961917564 [published Online First: 1996/05/01]
9. Bleeker-Rovers CP, Vos FJ, de Kleijn EM, et al. A prospective multicenter study on fever of unknown origin: the yield of a structured diagnostic protocol. *Medicine (Baltimore)* 2007;86(1):26-38. doi: 10.1097/MD.0b013e31802fe858 [published Online First: 2007/01/16]
10. Knockaert DC, Vanderschueren S, Blockmans D. Fever of unknown origin in adults: 40 years on. *J Intern Med* 2003;253(3):263-75. [published Online First: 2003/02/27]
11. Kucukardali Y, Oncul O, Cavuslu S, et al. The spectrum of diseases causing fever of unknown origin in Turkey: a multicenter study. *Int J Infect Dis* 2008;12(1):71-9. doi: 10.1016/j.ijid.2007.04.013 [published Online First: 2007/07/17]
12. Balink H, Verberne HJ, Bennink RJ, et al. A Rationale for the Use of F18-FDG PET/CT in Fever and Inflammation of Unknown Origin. *Int J Mol Imaging* 2012;2012:165080. doi: 10.1155/2012/165080 [published Online First: 2013/01/15]
13. Takeda R, Mizooka M, Kobayashi T, et al. Key diagnostic features of fever of unknown origin: Medical history and physical findings. *J Gen Fam Med* 2017;18(3):131-34. doi: 10.1002/jgf2.35 [published Online First: 2017/12/22]
14. Kim YS, Kim KR, Kang JM, et al. Etiology and clinical characteristics of fever of unknown origin in children: a 15-year experience in a single center. *Korean J Pediatr* 2017;60(3):77-85. doi: 10.3345/kjp.2017.60.3.77 [published Online First: 2017/04/11]
15. Cho CY, Lai CC, Lee ML, et al. Clinical analysis of fever of unknown origin in children: A 10-year experience in a northern Taiwan medical center. *J Microbiol Immunol Infect* 2017;50(1):40-45. doi: 10.1016/j.jmii.2015.01.001 [published Online First: 2015/03/05]
16. Tal S, Guller V, Gurevich A. Fever of unknown origin in older adults. *Clin Geriatr Med* 2007;23(3):649-68, viii. doi: 10.1016/j.cger.2007.03.004 [published Online First: 2007/07/17]
17. Simon L, Gauvin F, Amre DK, et al. Serum procalcitonin and C-reactive protein levels as markers of bacterial infection: a systematic review and meta-analysis. *Clin Infect Dis* 2004;39(2):206-17. doi: 10.1086/421997 [published Online First: 2004/08/13]

18. Kubota K, Nakamoto Y, Tamaki N, et al. FDG-PET for the diagnosis of fever of unknown origin: a Japanese multi-center study. *Ann Nucl Med* 2011;25(5):355-64. doi: 10.1007/s12149-011-0470-6 [published Online First: 2011/02/24]
19. Kouijzer IJ, Bleeker-Rovers CP, Oyen WJ. FDG-PET in fever of unknown origin. *Semin Nucl Med* 2013;43(5):333-9. doi: 10.1053/j.semnuclmed.2013.04.005 [published Online First: 2013/08/03]
20. Kouijzer IJE, Mulders-Manders CM, Bleeker-Rovers CP, et al. Fever of Unknown Origin: the Value of FDG-PET/CT. *Semin Nucl Med* 2018;48(2):100-07. doi: 10.1053/j.semnuclmed.2017.11.004 [published Online First: 2018/02/18]
21. Takeuchi M, Dahabreh IJ, Nihashi T, et al. Nuclear Imaging for Classic Fever of Unknown Origin: Meta-Analysis. *J Nucl Med* 2016;57(12):1913-19. doi: 10.2967/jnumed.116.174391 [published Online First: 2016/06/25]
22. Unger M, Karanikas G, Kerschbaumer A, et al. Fever of unknown origin (FUO) revised. *Wien Klin Wochenschr* 2016;128(21-22):796-801. doi: 10.1007/s00508-016-1083-9 [published Online First: 2016/09/28]
23. Knockaert DC, Vanneste LJ, Bobbaers HJ. Fever of unknown origin in elderly patients. *J Am Geriatr Soc* 1993;41(11):1187-92. [published Online First: 1993/11/01]
24. Sudo K, Kobayashi J, Noda S, et al. Japan's healthcare policy for the elderly through the concepts of self-help (Ji-jo), mutual aid (Go-jo), social solidarity care (Kyo-jo), and governmental care (Ko-jo). *Biosci Trends* 2018;12(1):7-11. doi: 10.5582/bst.2017.01271 [published Online First: 2018/02/27]
25. Iikuni Y, Okada J, Kondo H, et al. Current fever of unknown origin 1982-1992. *Intern Med* 1994;33(2):67-73. [published Online First: 1994/02/01]
26. Shoji S, Imamura A, Imai Y, et al. Fever of unknown origin: a review of 80 patients from the Shin'etsu area of Japan from 1986-1992. *Intern Med* 1994;33(2):74-6. [published Online First: 1994/02/01]
27. Ren J, Ma R, Zhang ZB, et al. Effects of microRNA-330 on vulnerable atherosclerotic plaques formation and vascular endothelial cell proliferation through the WNT signaling pathway in acute coronary syndrome. *Journal of Cellular Biochemistry* 2018;119(6):4514-27. doi: 10.1002/jcb.26584
28. He W, Goodkind D, Kowal P. U.S. Census Bureau, International Population Reports, P95/16-1, An Aging World: 2015. Government Publishing Office, Washington, DC. , 2016:1-165.
29. Hersch EC, Oh RC. Prolonged febrile illness and fever of unknown origin in adults. *Am Fam Physician* 2014;90(2):91-6. [published Online First: 2014/08/01]
30. Mourad O, Palda V, Detsky AS. A comprehensive evidence-based approach to fever of unknown origin. *Arch Intern Med* 2003;163(5):545-51. [published

Online First: 2003/03/08]
31. Mulders-Manders CM, Engwerda C, Simon A, et al. Long-term prognosis, treatment, and outcome of patients with fever of unknown origin in whom no diagnosis was made despite extensive investigation: A questionnaire based study. *Medicine (Baltimore)* 2018;97(25):e11241. doi: 10.1097/MD.0000000000011241 [published Online First: 2018/06/21]
32. Pedersen TI, Roed C, Knudsen LS, et al. Fever of unknown origin: a retrospective study of 52 cases with evaluation of the diagnostic utility of FDG-PET/CT. *Scand J Infect Dis* 2012;44(1):18-23. doi: 10.3109/00365548.2011.603741 [published Online First: 2011/09/06]
33. Robine A, Hot A, Maucort-Boulch D, et al. Fever of unknown origin in the 2000s: evaluation of 103 cases over eleven years. *Presse Med* 2014;43(9):e233-40. doi: 10.1016/j.lpm.2014.02.026 [published Online First: 2014/07/06]
34. Vanderschueren S, Del Biondo E, Ruttens D, et al. Inflammation of unknown origin versus fever of unknown origin: two of a kind. *Eur J Intern Med* 2009;20(4):415-8. doi: 10.1016/j.ejim.2009.01.002 [published Online First: 2009/06/16]

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).

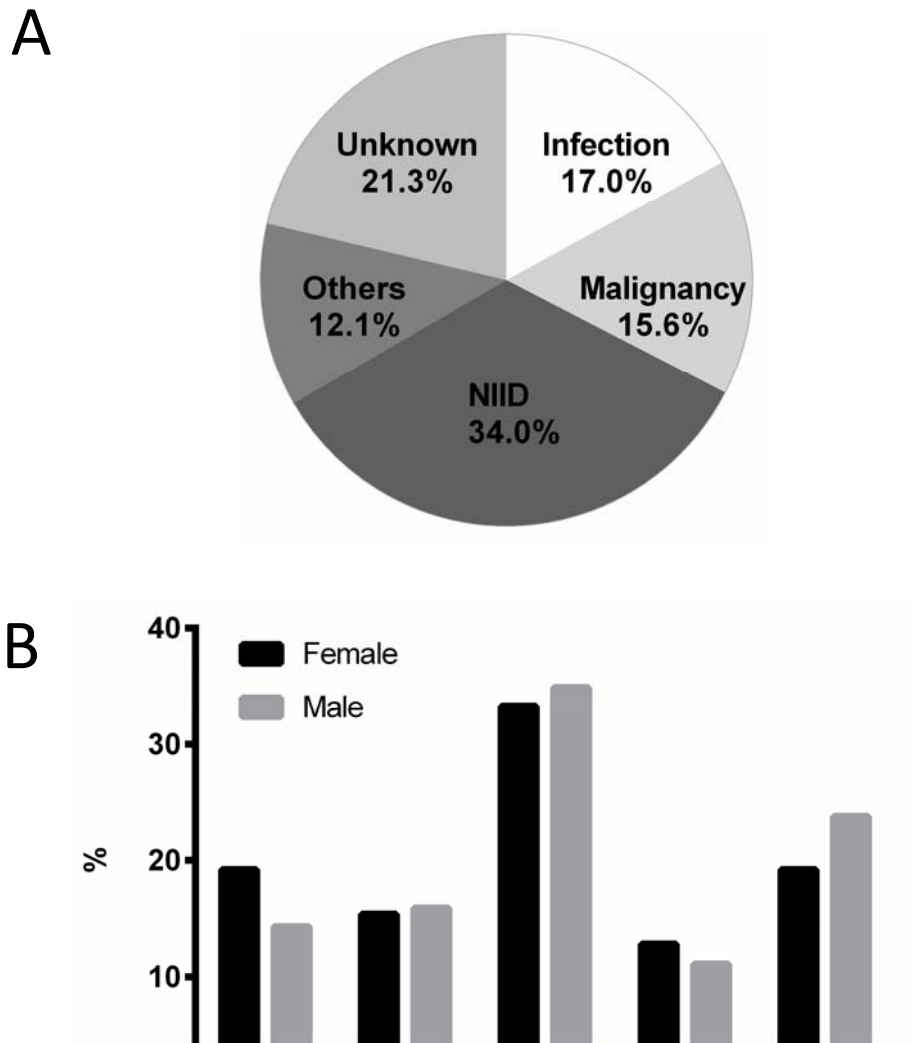
A
Unknown
21.3%
Infection
17.0%
Others
12.1%
Malignancy
15.6%
NIID
34.0%
B
Female
Male
%
40
30
20
10
0
Infection
Malignancy
NIID
Others
Unknown
C
<65 years
≥65 years
%
50
40
30
20
10
0
Infection
Malignancy
NIID
Others
Unknown

Figure 1

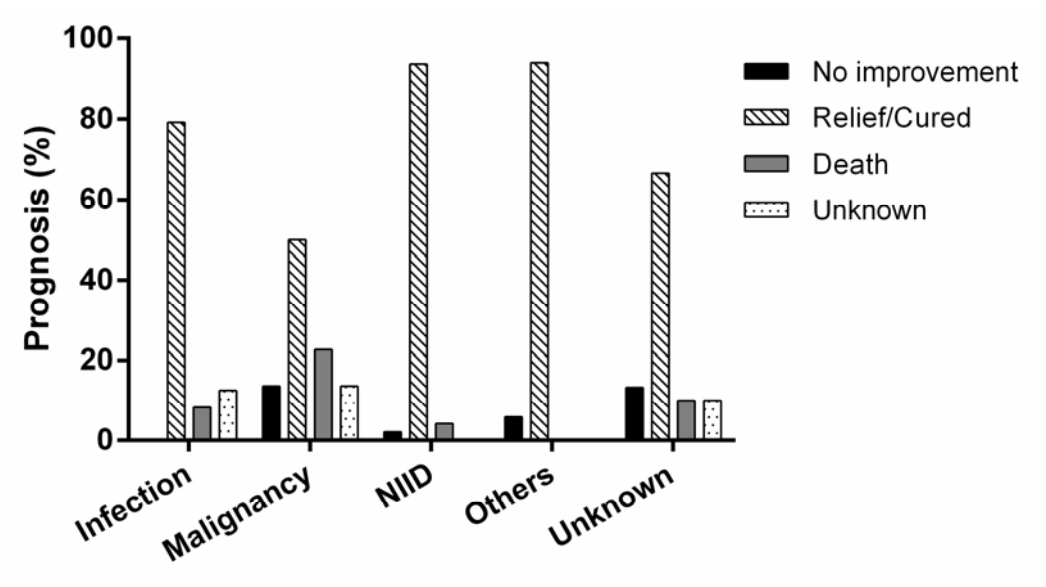
100
80
60
40
20
0
Prognosis (%)
No improvement
Relief/Cured
Death
Unknown
Infection
Malignancy
NIID
Others
Unknown

Figure 2

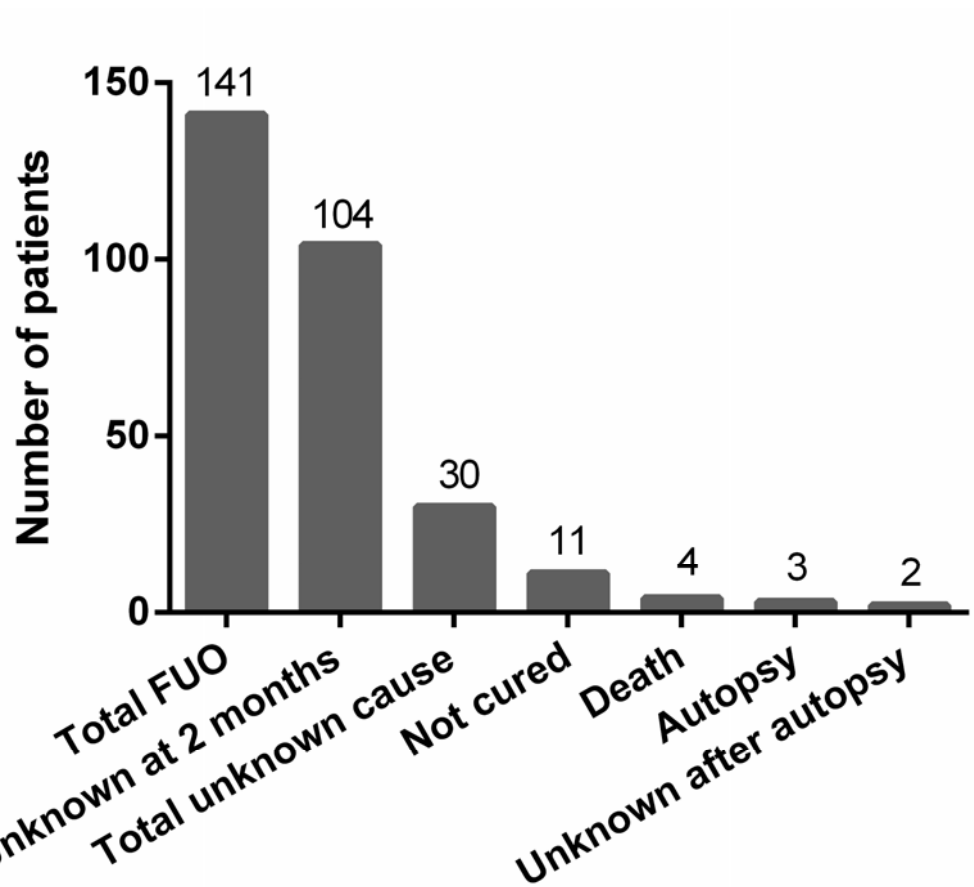
Number of patients
150
100
50
0
141
104
30
11
4
3
2
Total FUO
Unknown at 2 months
Total unknown cause
Not cured
Death
Autopsy
Unknown after autopsy

Figure 3

Supplementary

Supplementary Figure

Figure S1

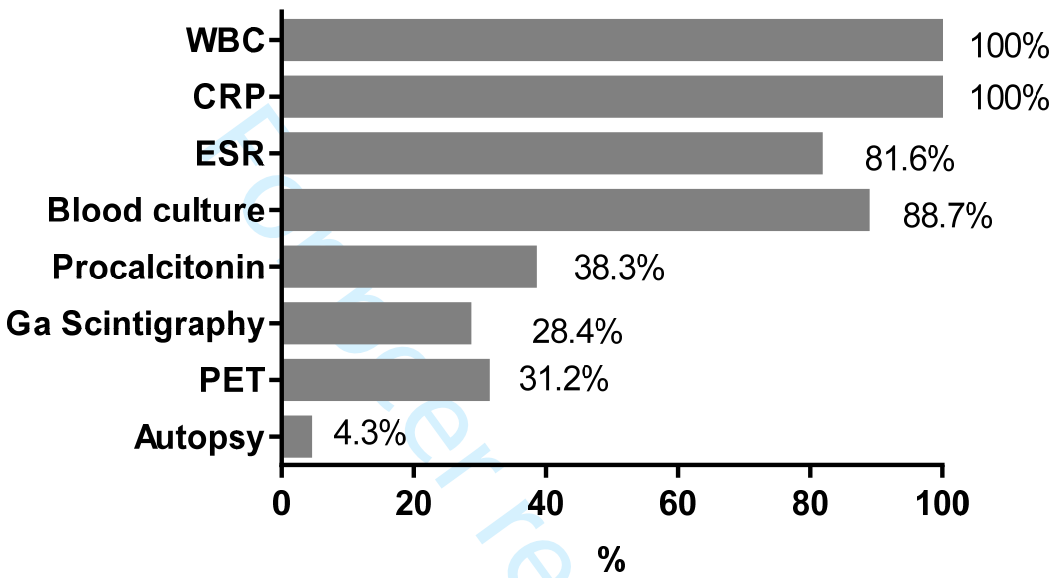

Figure S1. Frequency of examination for diagnostic evaluation. WBC, white blood cells count; CRP, C reactive protein; ESR, erythrocyte sedimentation rate; Ga, gallium; PET, positron emission tomography

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

	Time interval (days)		
Final diagnosis	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[a]		Final diagnosis					
		Total	Infection[b]	Malignancy[b]	NIID[b]	Others[b]	Unknown[b]
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[c]	Yes	141	24 (17.0%)	22 (15.6%)	48 (34.0%)	17 (12.1%)	30 (21.3%)
CRP[c]	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.

[a]Missing data would not be reported.

[b]Percentage was calculated as number of patients who performed examination divided by total patients for each condition.

[c]All patients performed examination of WBC and CRP.

Table S4. Distribution of examinations performed for diagnostic evaluation.

		Final diagnosis					
Examination[a]		Total	Infection[b]	Malignancy[b]	NIID[b]	Others[b]	Unknown[b]
WBC[c]	Yes	141	24 (17.0%)	22 (15.6%)	48 (34.0%)	17 (12.1%)	30 (21.3%)
CRP[c]	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.

[a]Missing data would not be reported.

[b]Percentage was calculated as number of patients who performed examination divided by total patients for each condition.

[c]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
Introduction			
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
Methods			
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*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—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 *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	7
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—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*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	n/a

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	n/a
Results			
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) *Cohort study*—Summarise follow-up time (eg, average and total amount)	9
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	10-11, Figures, sup tables
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	n/a
		Cross-sectional study—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
Discussion			
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
Other information			
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.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE 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 http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

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

Journal:	*BMJ Open*
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 Mediciene 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
Primary Subject Heading:	Geriatric medicine
Secondary Subject Heading:	Epidemiology, Diagnostics, Geriatric medicine
Keywords:	fever of unknown origin, elderly, erythrocyte sedimentation rate, prospective studies, aging population, Japan

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

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 ≥20 years diagnosed with classic FUO (axillary temperature ≥38.0°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±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

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

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

Key words: fever of unknown origin, elderly, erythrocyte sedimentation rate, prospective studies, aging population, Japan

Strengths and Limitations of this study

- This is the largest multicenter prospective study of fever of unknown origin (FUO) in Japanese hospitals.
- The locations of the hospitals involved are geographically dispersed across the country, covering the eastern and western regions of Japan, representing the largest FUO data in Japan.

- Key diagnostic features and the causes of FUO were analyzed with respect to patients' medical histories, physical examination findings, blood tests and imaging.
- The study included the characteristics of FUO cases whose causative disease remained unknown after clinical investigation.
- Our study identified age and ESR test as potentially important factors useful in assisting clinicians seeking to reveal the causes of FUO.

INTRODUCTION

Fever of unknown origin (FUO) has many possible causes which can vary depending on region and time period.[1-3] FUO was first described in the medical literature in 1930[4] and defined in 1961[5] Since then, a significantly changing spectrum of diseases causing FUO has been reported.[6-12] The causes of FUO have now been classified as infections, non-infectious inflammatory diseases (NIID), malignancies, other conditions and unknown.[1 3] The proportion of different causative diseases of FUO has changed over time,[13] with fewer cases of FUO caused by infections and neoplasms over the past 40-50 years.[14] NIID is now the most common cause of FUO in adults,[1 15] while infectious diseases are most common in children.[16 17] In recent studies from Europe and the United States, the percentage of patients with unknown FUO varied from 7% to 53%.[9] 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.[2 3 18] Most previous studies of FUO have focused on its etiology and prevalence,[3] outcomes or the diagnostic value of such tools as inflammatory markers[19 20] or positron emission tomography (PET).[21-24] However, limited studies have assessed the clinical utility of standard inflammatory markers, even

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

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

PATIENTS AND METHODS

Patients

This prospective study assessed patients aged ≥20 years with classic FUO from 16

hospitals (encompassing the eastern and western regions of Japan) affiliated with the Japanese Society of Hospital General Medicine, between January 2016 and December 2017. Classic FUO was diagnosed based on the definition used in Naito et al.[1] in patients meeting all of the following criteria: 1) fever ≥38.0°C at least twice within a 3-week period; 2) unknown etiology of fever after three outpatient visits or 3 days of hospitalization; and 3) no diagnosis of immunodeficiency or confirmed human immunodeficiency virus (HIV) infection prior to fever onset.

The following data from patients were collected during a 6-month follow-up period and recorded on standardized case report forms: patient characteristics (sex, age, comorbidities, medical history and symptoms); physical examination; blood tests (blood count, general biochemical tests, inflammatory markers: erythrocyte sedimentation rate [ESR], C-reactive protein [CRP] level, procalcitonin level); results of blood culture if performed; results of imaging studies and endoscopy if performed; results of cytology, histology and genetic testing, or autopsy findings if performed; and final diagnosis, day of diagnosis and follow-up diagnosis outcome. In addition to analyzing the frequency of different causative diseases and outcomes of FUO cases, we evaluated the association between the presence or absence of examination for diagnostic evaluation, the number of days to diagnosis and the clinical follow-up results of inflammatory markers and other imaging tests.

Final diagnoses of the cause of FUO were classified into: infections, NIID, malignancies, other conditions and unknown. Unknown was defined as having no definitive diagnosis after 6 months of clinical investigation.

Statistical Analysis

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

Patient and public involvement statement

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.

RESULTS

Patient characteristics

A total of 141 patients who met the criteria of FUO were prospectively recruited from 16 hospitals, including 78 females (55.3%) and 63 males (44.7%), with a median age

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

Figure 1B and C show the distribution of the final diagnosis of FUO by sex and age. The final diagnosis of FUO had no significant correlation with sex (**Fig 1B;** $\chi 2=1.0$, df=4, p=0.916) but there was a significant correlation with age (**Fig 1C**; $\chi 2=9.7$, df=4, p=0.046). NIIDs constituted the major cause among patients aged ≥65 years (43.1%) and those <65 years (26.3 %). A lower percentage of patients aged ≥65 years (4.6%)

were diagnosed with other causative diseases compared to those aged <65 years (18.4%).

Symptoms and signs

The comorbidities and symptoms in FUO patients by final diagnosis are presented in **Table 2**. Comorbidities included chronic conditions such as hypertension, diabetes and dyslipidemia. A much higher percentage of patients with comorbidities were diagnosed with malignant neoplasm than those without (19.3% vs. 9.6%). The major cause of FUO in patients without comorbidities was NIID (40.4%). Higher percentages of patients with respiratory (33.3%) and gastrointestinal (23.8%) symptoms were diagnosed with infectious diseases. Furthermore, the cause of FUO was NIID in most patients with symptoms of arthralgia (61.4%) or muscle pain (63.2%).

Biochemical and imaging results

White blood cells (WBC) and CRP were examined in all patients, while 81.6% of patients were tested for ESR and 88.7% for blood culture **(Fig S1).** Only 38.3% of patients had procalcitonin tests. One in four or five patients underwent imaging scans (28.4% for Gallium Scintigraphy and 31.2% for PET). Autopsy was performed in only 4.3% of patients. Patients who underwent an ESR test had a greater likelihood of being diagnosed with a malignant neoplasm (17.4%) or unknown cause (25.2%) compared to those without an ESR test. Patients who had undergone an imaging examination had a

relatively greater likelihood of being diagnosed with malignancy or NIID compared to those without imaging examinations (**Table 2**).

There was a significant association between the etiology of FUO and the prognosis of patients (**Fig 2**; $\chi 2=27.6$, df=12, p=0.006). Most patients with FUO with different causative diseases generally were cured or experienced relief. However, patients with malignancy or unknown causes had higher mortality rates (22.7% and 12.9%, respectively) (Figure 2). Among all 141 patients, the cause of fever was not identified in 104 patients at 2 months (**Fig 3**). At the end of the follow-up period, the cause of FUO remained unknown in 30 patients and 11 patients were not cured or had no symptom relief. Four deaths occurred among these patients. Pathological autopsy was performed on a small proportion of those who died (n=3); two cases remained unknown after autopsy (**Fig 3**).

Tests were performed for diagnostic evaluation and abnormal readings were defined as in Naito et al.:[1] WBC: 4000-8000; CRP: 0.3; ESR >100 mm/hr and procalcitonin ≥0.25 ng/mL. Most patients with unknown cause of FUO had abnormal WBC and CRP levels (WBC: 56.7%; CRP: 73.3%, respectively) while a smaller percentage of patients had abnormal ESR and procalcitonin levels (ESR: 24.1%; procalcitonin: 23.1%). **Table 3** shows the association of patient demographics, clinical characteristics and diagnostic examinations for patients with known and unknown causes of FUO. There was a

significant association between having undergone ESR examination and unknown final diagnosis of FUO (odds ratio=8.43, 95% confidence interval=1.09-65.00, p=0.041). Furthermore, 80% (28 of 35) of patients with an abnormal ESR value had a final diagnosis. No other variables differed significantly between the groups with known and unknown cause of FUO (all p>0.05) (**Table 3**).

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[a]		Total	Final diagnosis				
			Infection[b]	Malignancy[b]	NIID[b]	Other[b]	Unknown[b]
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 (9.1%)	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[c]	Yes	141	24 (17.0%)	22 (15.6%)	48 (34.0%)	17 (12.1%)	30 (21.3%)
CRP[c]	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.

[a]Missing data would not be reported.

[b]Percentage was calculated as number of patients who performed examination divided by total patients for each condition.

[c]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)	0.566
	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)	0.425
	No	97 (77.6%)	28 (22.4%)	1.00	
Rash	Yes	26 (81.3%)	6 (18.8%)	0.82 (0.30-2.21)	0.692
	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	

[a]Percentage was calculated as the number of patients who received an examination divided by the total patients for each condition.

[b]Chi-square tests were performed.

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

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

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 (≥65 and <65 years), but not with sex. While most studies have identified NIID as the most common cause of FUO in Japan,[1 15 28 29] our 2013 study found similar rates of NIID as a cause of FUO in participants ≥65 and <65 years.[3] 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 ≥65 accounted for 26.7% of the 127.11 million population in 2016,[27 30] and will increase to 40% in 2050, according to a new analysis.[31] In this study, 46.1% of patients were ≥65 years, an increase since 2013 (42.1%).[3] Moreover, this trend should also be considered in Western countries, where aging of the population is also expected.[31] A diagnosis of NIID, which occurs significantly more often in elderly patients,[1] consequently must be considered first for an FUO, particularly in patients ≥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

population depends on the genotype combinations of the HLD DRB1 and DQB1 alleles, and predisposing risk has been found associated with the haplotype DRB1*15:01-DQB1*06:02 in Japanese patients with AOSD.[32] However, genotyping results were not available for this study.

Difference in causative disease between populations could be influenced by factors such as geographic location, zoonotic characteristics and the economic and medical organization of the local health care system. Infectious disease was the leading cause of FUO in South-East Europe, as reported by Baymakova et al. in 2016.[33] Infection was the second the most common causes of fever in our patient population. Our previous study in 2013 demonstrated that PMR and HIV should be considered as causes of FUO.[3] However, HIV was not found in this study, possibly due to the efficiency of HIV testing in Japan. The frequency of unknown cause in our study was comparable to that found previously in 2013.[3]

The availability of new diagnostic techniques, including computed tomography (CT), PET imaging, improved culture techniques and advanced serologic assays has changed both the spectrum of diseases causing FUO and the time to reveal the final diagnosis. In a previous study, the cause of FUO diagnosed after ≥100 days was malignancy.[3] In this study, more than 50% of FUO patients with infections, malignancy, NIID and other causes had a final diagnosis within 100 days of fever onset. Similarly,

in a series of patients with FUO studied in Europe and USA, 30-50% were of unknown cause after a follow-up of ≥100 days.[6 9 34]

In the present study, we evaluated key symptoms and signs in patients with FUO, to determine which were diagnostically useful. We found that comorbidities were the main symptoms and signs in FUO caused by malignant neoplasms. Patients with infectious diseases often had respiratory and gastrointestinal symptoms, while those with NIID often had arthralgia or muscle pain. Although the various symptoms/signs were not directly related to the final diagnosis of FUO,[14] their presence might help improve the differential diagnosis in patients with FUO.

A systemic review from 2003 reported that the prevalence of FUO was 1.5-3% in all hospitalized patients, and mortality in these patients was 12-35%.[35] We found that the etiology of FUO was significantly associated with prognosis; FUO patients diagnosed with malignancy or unknown causes had higher mortality rates. A Danish study also found that FUO patients with malignancy had poor prognosis.[36] Little is known about the prognosis of patients with FUO of unknown cause. In our study, 4 of 30 (13.3%) patients with FUO of unknown cause died during within 6 months; the cause of FUO remained unknown after autopsy in two of these patients. In patients with FUO of unknown cause, Dutch studies showed mortality rates of 2.0-4.0%[6 36] and other western-European studies reported mortality rates of 2.0-19.0%.[7 10 37-39] The variances

among studies may be due to differences in patient selection, study design or health care systems.

Since there is no standard diagnostic approach in FUO, classic test features are difficult to apply in FUO studies. Of all positive biochemical tests, only 1.7% contributed indirectly to diagnosis in a Turkey FUO study.[13] Despite advances in diagnostic tests and techniques, a significant proportion of all cases remains undiagnosed.[40] Our previous study found that 14.9% of FUO patient had an ESR >100 mm/hr, including 5 with FUO of unknown cause[1]. In the current study, 35 of 115 patients (30.4%) had an abnormal ESR test result; in these, the cause of FUO was identified in 80% of patients. In addition, there was a significant association between known cause and ancillary ESR test, but not with other variables such as procalcitonin or PET. Therefore, the current study demonstrated the usefulness of ESR in evaluating FUO. However, further investigation is required. We speculate that future FUO research may be leaving the twilight zone as diagnostic microcellular research technologies emerge from the laboratory to point-of-care rapid diagnostic kits. We await further advances in diagnostic artificial intelligence to expose FUO cause in more cases.[41 42]

The present study has the following limitations. First, despite this being the largest data sample ever collected from geographically-dispersed Japanese hospitals, the

sample size is still small; caution should be taken when generalizing our results. Also, we did not establish uniformity of the diagnostic criterion used in this study, which may have resulted in over- or under-diagnosis of specific disease categories. Uncertainty of diagnosis was not addressed. Finally, our follow-up database was not designed to include records of spontaneous fever remission.

In conclusion, evaluating and determining the cause of a fever is complex. The availability of new diagnostic techniques (including CT and PET imaging), improved culture techniques and recent advances in serologic assays have all changed both the spectrum of diseases causing FUO and the time needed to reach a final diagnosis. Our study identified age and ESR as potentially important factors useful in assisting clinicians navigate the paths to diagnosing FUO. These advances, together with future development of multi-microbial and cancer cell detection tools, may allow faster determination of the causes of FUO and further improve the prognosis of FUO patients.

Funding:

This work was supported by Grants-in-Aid for Scientific Research, Japan Project Number: 16K09257.

Competing interests:

None

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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Acknowledgments:

We would like to acknowledge Convergence CT Japan K.K for conducting statistical

analysis, data interpretation, and reviewing this manuscript. We would also like to acknowledge support from Tatsuo Ishizuka, Kenji Kanazawa, Nobuki Nanki, Megumi Higaki, and Koichi Mashiba for valuable administrative and research assistance throughout this study.

Ethics approval:

Research Ethics Committee of Juntendo University School of Medicine

Data availability statement:

All data generated within this study are available from the corresponding author on request.

REFERENCES

1. Naito T, Torikai K, Mizooka M, et al. Relationships between causes of fever of unknown origin and inflammatory markers: A multicenter collaborative retrospective study *Intern Med*2015;**54**:1989-94.doi:10.2169/internalmedicine.54.3313

2. Cunha BA, Lortholary O, Cunha CB. Fever of unknown origin: a clinical approach. *Am J Med*2015;**128**:1138.doi:10.1016/j.amjmed.2015.06.001

3. Naito T, Mizooka M, Mitsumoto F, et al. Diagnostic workup for fever of unknown origin: a multicenter collaborative retrospective study. *BMJ Open*2013;**3**:e003971.doi:10.1136/bmjopen-2013-003971

4. Alt HL, Barker MH. Fever of unknown origin. *JAMA*1930;**94**:1457–61

5. Petersdorf RG, Beeson PB. Fever of unexplained origin: report on 100 cases. *Medicine (Baltimore)*1961;**40**:1-30

6. de Kleijn EM, Vandenbroucke JP, van der Meer JW. Fever of unknown origin (FUO). I A. prospective multicenter study of 167 patients with FUO, using fixed epidemiologic entry criteria. The Netherlands FUO Study Group. *Medicine (Baltimore)*1997;**76**:392-400

7. Zenone T. Fever of unknown origin in adults: evaluation of 144 cases in a non-university hospital. *Scand J Infect Dis*2006;**38**:632-8. doi:10.1080/00365540600606564

8. Miller RF, Hingorami AD, Foley NM. Pyrexia of undetermined origin in patients with human immunodeficiency virus infection and AIDS. *Int J STD AIDS*1996;**7**:170-5.doi:10.1258/0956462961917564

9. Bleeker-Rovers CP, Vos FJ, de Kleijn EM, et al. A prospective multicenter study on fever of unknown origin: the yield of a structured diagnostic protocol. *Medicine (Baltimore)*2007;**86**:26-38.doi:10.1097/MD.0b013e31802fe858

10. Knockaert DC, Vanderschueren S, Blockmans D. Fever of unknown origin in adults: 40 years on. *J Intern Med*2003;**253**:263-75

11. Durack DT, Street AC. Fever of unknown origin--reexamined and redefined. *Curr Clin Top Infect Dis*1991;**11**:35-51

12. Baymakova M, Popov GT, Andonova R, et al. Fever of unknown origin and Q-

fever: a case series in a Bulgarian hospital. *Caspian J Intern Med*2019;**10**:102-06.doi:10.22088/cjim.10.1.102

13. Kucukardali Y, Oncul O, Cavuslu S, et al. The spectrum of diseases causing fever of unknown origin in Turkey: a multicenter study. *Int J Infect Dis*2008;**12**:71-9.doi:10.1016/j.ijid.2007.04.013

14. Balink H, Verberne HJ, Bennink RJ, et al. A Rationale for the use of F18-FDG PET/CT in fever and inflammation of unknown origin. *Int J Mol Imaging*2012;**2012**:165080.doi:10.1155/2012/165080

15. Takeda R, Mizooka M, Kobayashi T, et al. Key diagnostic features of fever of unknown origin: Medical history and physical findings. *J Gen Fam Med*2017;**18**:131-34.doi:10.1002/jgf2.35

16. Kim YS, Kim KR, Kang JM, et al. Etiology and clinical characteristics of fever of unknown origin in children: a 15-year experience in a single center. *Korean J Pediatr*2017;**60**:77-85.doi:10.3345/kjp.2017.60.3.77

17. Cho CY, Lai CC, Lee ML, et al. Clinical analysis of fever of unknown origin in children: A 10-year experience in a northern Taiwan medical center. *J Microbiol Immunol Infect*2017;**50**:40-45.doi:10.1016/j.jmii.2015.01.001

18. Tal S, Guller V, Gurevich A. Fever of unknown origin in older adults. *Clin Geriatr Med*2007;**23**:649-68, viii.doi:10.1016/j.cger.2007.03.004

19. Simon L, Gauvin F, Amre DK, et al. Serum procalcitonin and C-reactive protein levels as markers of bacterial infection: a systematic review and meta-analysis. *Clin Infect Dis*2004;**39**:206-17.doi:10.1086/421997

20. Kubota K, Nakamoto Y, Tamaki N, et al. FDG-PET for the diagnosis of fever of unknown origin: a Japanese multi-center study. *Ann Nucl Med*2011;**25**:355-64.doi:10.1007/s12149-011-0470-6

21. Kouijzer IJ, Bleeker-Rovers CP, Oyen WJ. FDG-PET in fever of unknown origin. *Semin Nucl Med*2013;**43**:333-9.doi:10.1053/j.semnuclmed.2013.04.005

22. Kouijzer IJE, Mulders-Manders CM, Bleeker-Rovers CP, et al. Fever of unknown origin: the value of FDG-PET/CT. *Semin Nucl Med*2018;**48**:100-07.doi:10.1053/j.semnuclmed.2017.11.004

23. Takeuchi M, Dahabreh IJ, Nihashi T, et al. Nuclear imaging for classic fever of

unknown origin: Meta-analysis. *J Nucl Med*2016;**57**:1913-19.doi:10.2967/jnumed.116.174391

24. Bleeker-Rovers CP. Positron emission tomography with 18F-fluorodeoxyglucose in fever of unknown origin and infectious and non-infectious inflammatory diseases. *Radboud University Nijmegen, Netherlands*2007

25. Unger M, Karanikas G, Kerschbaumer A, et al. Fever of unknown origin (FUO) revised. *Wien Klin Wochenschr*2016;**128**:796-801.doi:10.1007/s00508-016-1083-9

26. Knockaert DC, Vanneste LJ, Bobbaers HJ. Fever of unknown origin in elderly patients. *J Am Geriatr Soc*1993;**41**:1187-92

27. Sudo K, Kobayashi J, Noda S, et al. Japan's healthcare policy for the elderly through the concepts of self-help (Ji-jo), mutual aid (Go-jo), social solidarity care (Kyo-jo), and governmental care (Ko-jo). *Biosci Trends*2018;**12**:7-11.doi:10.5582/bst.2017.01271

28. Iikuni Y, Okada J, Kondo H, et al. Current fever of unknown origin 1982-1992. *Intern Med*1994;**33**:67-73

29. Shoji S, Imamura A, Imai Y, et al. Fever of unknown origin: a review of 80 patients from the Shin'etsu area of Japan from 1986-1992. *Intern Med*1994;**33**:74-6

30. Ren J, Ma R, Zhang ZB, et al. Effects of microRNA-330 on vulnerable atherosclerotic plaques formation and vascular endothelial cell proliferation through the WNT signaling pathway in acute coronary syndrome. *Journal of Cellular Biochemistry*2018;**119**:4514-27.doi:10.1002/jcb.26584

31. He W, Goodkind D, Kowal P. U.S. Census Bureau, International Population Reports, P95/16-1, An Aging World: 2015. Government Publishing Office, Washington, DC. , 2016:1-165.

32. Fujita Y, Furukawa H, Asano T, et al. HLA-DQB1 DPB1 alleles in Japanese patients with adult-onset Still's disease. *Mod Rheumatol*2018:1-5.doi:10.1080/14397595.2018.1514999

33. Baymakova M PK, Dikov I, Popov GT, Mihaylova-Garnizova R, Kovaleva V, Kundurdjiev T. Fever of unknown origin in a Bulgarian hospital: evaluation of 54 cases for a four year-period. *J Clin Anal Med*2016;**7**:70-75.doi:10.4328/JCAM.3897

34. Hersch EC, Oh RC. Prolonged febrile illness and fever of unknown origin in

adults. *Am Fam Physician*2014;**90**:91-6

35. Mourad O, Palda V, Detsky AS. A comprehensive evidence-based approach to fever of unknown origin. *Arch Intern Med*2003;**163**:545-51

36. Mulders-Manders CM, Engwerda C, Simon A, et al. Long-term prognosis, treatment, and outcome of patients with fever of unknown origin in whom no diagnosis was made despite extensive investigation: A questionnaire based study. *Medicine(Baltimore)*2018;**97**:e11241.doi:10.1097/MD.0000000000011241

37. Pedersen TI, Roed C, Knudsen LS, et al. Fever of unknown origin: a retrospective study of 52 cases with evaluation of the diagnostic utility of FDG-PET/CT. *Scand J Infect Dis*2012;**44**:18-23.doi:10.3109/00365548.2011.603741

38. Robine A, Hot A, Maucort-Boulch D, et al. Fever of unknown origin in the 2000s: evaluation of 103 cases over eleven years. *Presse Med*2014;**43**:e233-40.doi:10.1016/j.lpm.2014.02.026

39. Vanderschueren S, Del Biondo E, Ruttens D, et al. Inflammation of unknown origin versus fever of unknown origin: two of a kind. *Eur J Intern Med*2009;**20**:415-8.doi:10.1016/j.ejim.2009.01.002

40. Horowitz HW. Fever of unknown origin or fever of too many origins? *N Engl J Med*2013;**368**:197-9.doi:10.1056/NEJMp1212725

41. Dave VP, Ngo TA, Pernestig AK, et al. MicroRNA amplification and detection technologies: opportunities and challenges for point of care diagnostics. *Lab Invest*2019;**99**:452-69.doi:10.1038/s41374-018-0143-3

42. Liang H, Tsui BY, Ni H, et al. Evaluation and accurate diagnoses of pediatric diseases using artificial intelligence. *Nat Med*2019;**25**:433-38.doi:10.1038/s41591-018-0335-9

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).

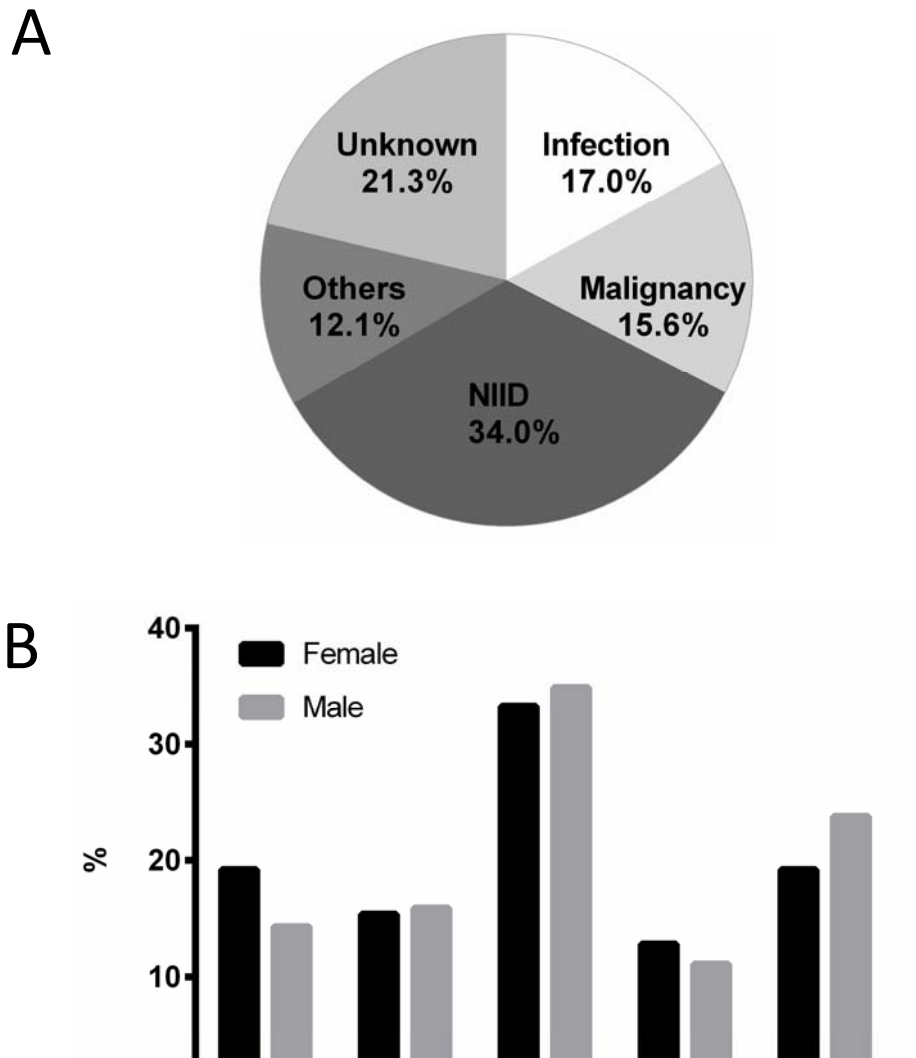
A
Unknown
21.3%
Infection
17.0%
Others
12.1%
Malignancy
15.6%
NIID
34.0%
B
Female
Male
%
40
30
20
10
0
Infection
Malignancy
NIID
Others
Unknown
C
<65 years
≥65 years
%
50
40
30
20
10
0
Infection
Malignancy
NIID
Others
Unknown

Figure 1

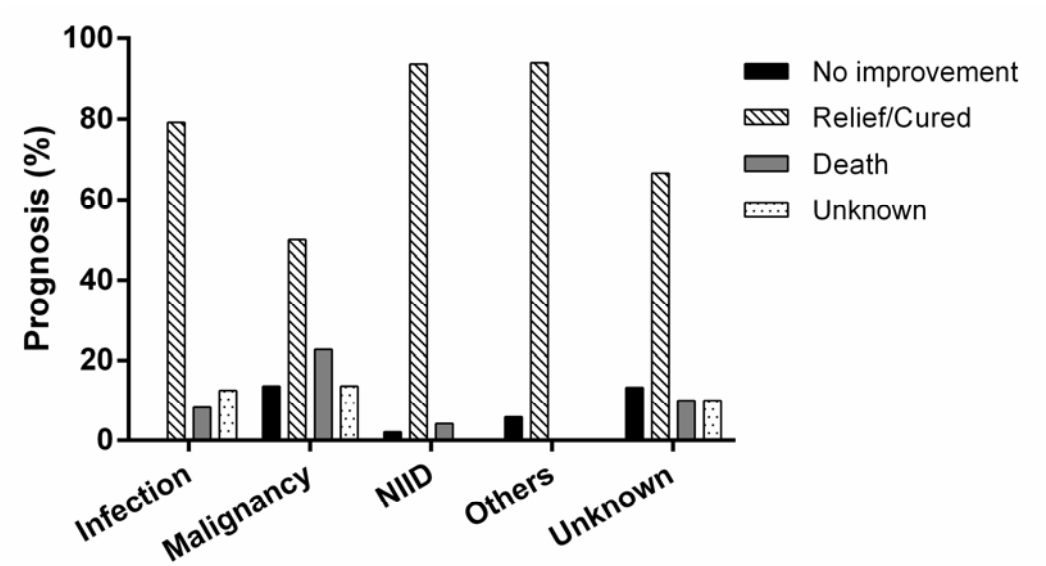
100
80
60
40
20
0
Prognosis (%)
Infection
Malignancy
NIID
Others
Unknown
No improvement
Relief/Cured
Death
Unknown

Figure 2

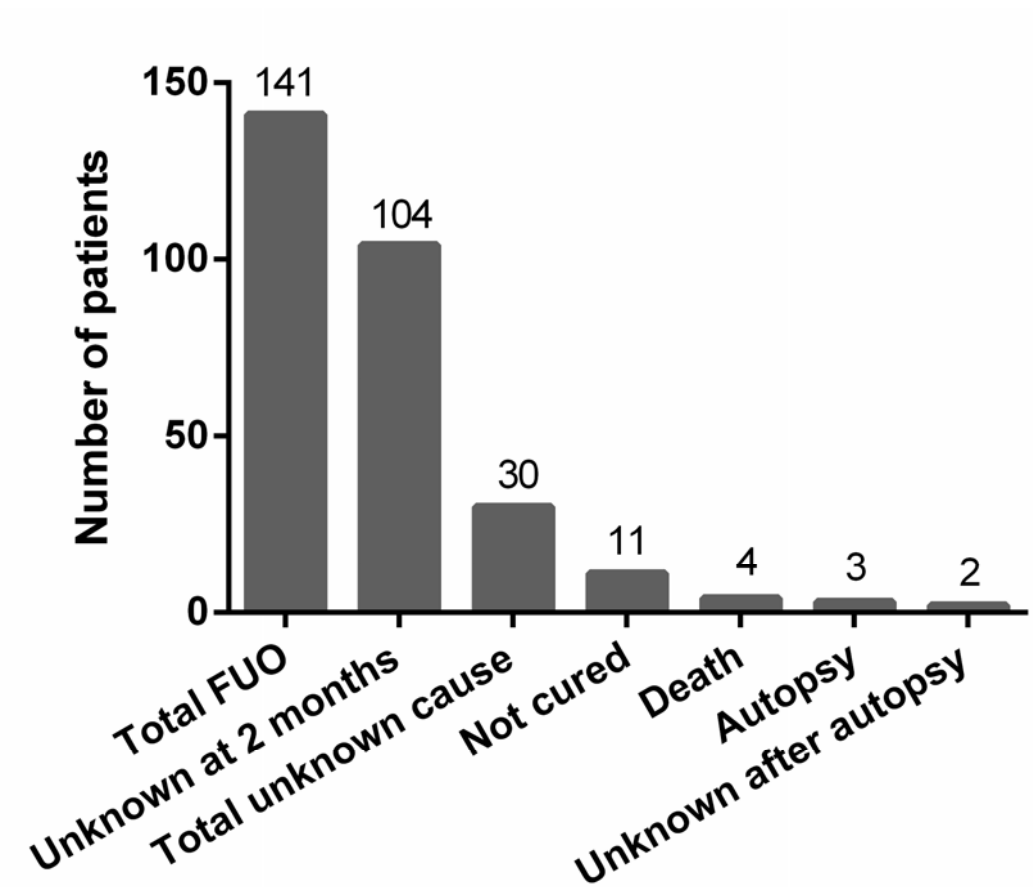
Number of patients
150
100
50
0
141
104
30
11
4
3
2
Total FUO
Unknown at 2 months
Total unknown cause
Not cured
Death
Autopsy
Unknown after autopsy

Figure 3

Supplementary

Supplementary Figure

Figure S1

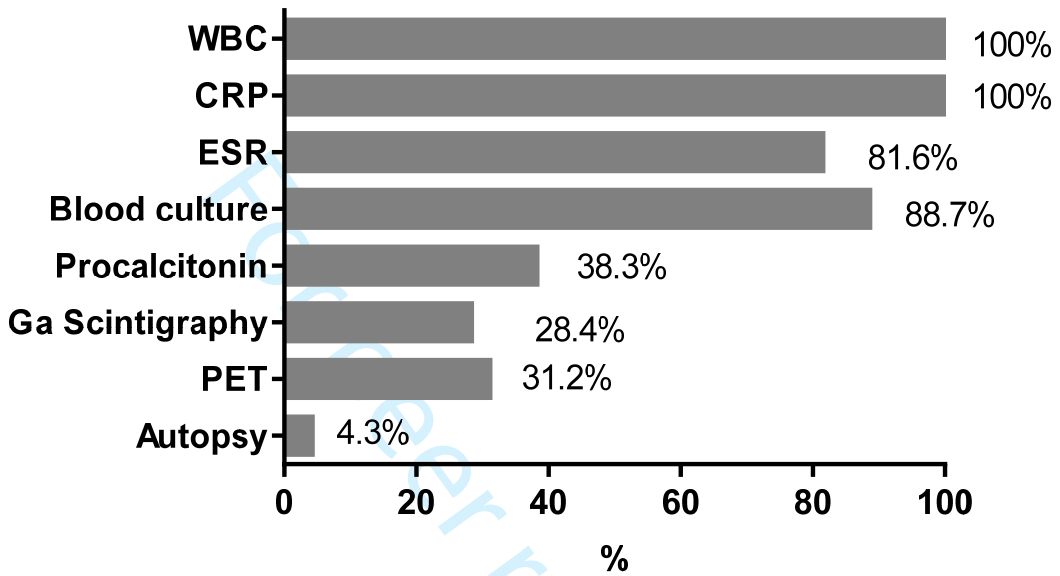

Figure S1. Frequency of examination for diagnostic evaluation. WBC, white blood cells count; CRP, C reactive protein; ESR, erythrocyte sedimentation rate; Ga, gallium; PET, positron emission tomography

Supplementary Tables

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

	Time interval (days)		
Final diagnosis	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
Introduction			
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
Methods			
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*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—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 *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	8
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—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*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	n/a

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	n/a
Results			
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) *Cohort study*—Summarise follow-up time (eg, average and total amount)	9
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	10-11, Figures, sup tables
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	n/a
		Cross-sectional study—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
Discussion			
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
Other information			
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.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE 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 http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

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

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-032059.R2
Article Type:	Original research
Date Submitted by the Author:	25-Oct-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 Mediciene 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
Primary Subject Heading:	Geriatric medicine
Secondary Subject Heading:	Epidemiology, Diagnostics, Geriatric medicine
Keywords:	fever of unknown origin, elderly, erythrocyte sedimentation rate, prospective studies, aging population, Japan

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

Running title: Diagnosis for fever of unknown origin

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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 ≥20 years diagnosed with classic FUO (axillary temperature ≥38.0°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±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

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

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

Key words: fever of unknown origin, elderly, erythrocyte sedimentation rate, prospective studies, aging population, Japan

Strengths and Limitations of this study

- This is the largest multicenter prospective study of fever of unknown origin (FUO) in Japanese hospitals.
- The locations of the hospitals involved are geographically dispersed across the country, covering the eastern and western regions of Japan, representing the largest FUO data in Japan.

- Key diagnostic features and the causes of FUO were analyzed with respect to patients' medical histories, physical examination findings, standard blood tests and imaging examinations.
- Despite this being the largest data sample collected from Japanese hospitals, the sample size is still small; caution should be taken when generalizing the results.

INTRODUCTION

Fever of unknown origin (FUO) has many possible causes which can vary depending on region and time period.[1-3] FUO was first described in the medical literature in 1930[4] and defined in 1961[5] Since then, a significantly changing spectrum of diseases causing FUO has been reported.[6-12] The causes of FUO have now been classified as infections, non-infectious inflammatory diseases (NIID), malignancies, other conditions and unknown.[1 3] The proportion of different causative diseases of FUO has changed over time,[13] with fewer cases of FUO caused by infections and neoplasms over the past 40-50 years.[14] NIID is now the most common cause of FUO in adults,[1 15] while infectious diseases are most common in children.[16 17] In recent studies from Europe and the United States, the percentage of patients with unknown FUO varied from 7% to 53%.[9] 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.[2 3 18] Most previous studies of FUO have focused on its etiology and prevalence,[3] outcomes or the diagnostic value of such tools as inflammatory markers[19 20] or positron emission tomography (PET).[21-24] However, limited studies have assessed the clinical utility of standard inflammatory markers, even

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

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

PATIENTS AND METHODS

Patients

This prospective study assessed patients aged ≥20 years with classic FUO from 16

hospitals (encompassing the eastern and western regions of Japan) affiliated with the Japanese Society of Hospital General Medicine, between January 2016 and December 2017. Classic FUO was diagnosed based on the definition used in Naito et al.[1] in patients meeting all of the following criteria: 1) fever ≥38.0°C at least twice within a 3-week period; 2) unknown etiology of fever after three outpatient visits or 3 days of hospitalization; and 3) no diagnosis of immunodeficiency or confirmed human immunodeficiency virus (HIV) infection prior to fever onset.

The following data from patients were collected during a 6-month follow-up period and recorded on standardized case report forms: patient characteristics (sex, age, comorbidities, medical history and symptoms); physical examination; blood tests (blood count, general biochemical tests, inflammatory markers: erythrocyte sedimentation rate [ESR], C-reactive protein [CRP] level, procalcitonin level); results of blood culture if performed; results of imaging studies and endoscopy if performed; results of cytology, histology and genetic testing, or autopsy findings if performed; and final diagnosis, day of diagnosis and follow-up diagnosis outcome. In addition to analyzing the frequency of different causative diseases and outcomes of FUO cases, we evaluated the association between the presence or absence of examination for diagnostic evaluation, the number of days to diagnosis and the clinical follow-up results of inflammatory markers and other imaging tests.

Final diagnoses of the cause of FUO were classified into: infections, NIID, malignancies, other conditions and unknown. Unknown was defined as having no definitive diagnosis after 6 months of clinical investigation.

Statistical Analysis

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

Patient and public involvement statement

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.

RESULTS

Patient characteristics

A total of 141 patients who met the criteria of FUO were prospectively recruited from 16 hospitals, including 78 females (55.3%) and 63 males (44.7%), with a median age

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

Figure 1B and C show the distribution of the final diagnosis of FUO by sex and age. The final diagnosis of FUO had no significant correlation with sex (**Fig 1B;** $\chi 2=1.0$, df=4, p=0.916) but there was a significant correlation with age (**Fig 1C**; $\chi 2=9.7$, df=4, p=0.046). NIIDs constituted the major cause among patients aged ≥65 years (43.1%) and those <65 years (26.3 %). A lower percentage of patients aged ≥65 years (4.6%)

were diagnosed with other causative diseases compared to those aged <65 years (18.4%).

Symptoms and signs

The comorbidities and symptoms in FUO patients by final diagnosis are presented in **Table 2**. Comorbidities included chronic conditions such as hypertension, diabetes and dyslipidemia. A much higher percentage of patients with comorbidities were diagnosed with malignant neoplasm than those without (19.3% vs. 9.6%). The major cause of FUO in patients without comorbidities was NIID (40.4%). Higher percentages of patients with respiratory (33.3%) and gastrointestinal (23.8%) symptoms were diagnosed with infectious diseases. Furthermore, the cause of FUO was NIID in most patients with symptoms of arthralgia (61.4%) or muscle pain (63.2%).

Biochemical and imaging results

White blood cells (WBC) and CRP were examined in all patients, while 81.6% of patients were tested for ESR and 88.7% for blood culture **(Fig S1).** Only 38.3% of patients had procalcitonin tests. One in four or five patients underwent imaging scans (28.4% for Gallium Scintigraphy and 31.2% for PET). Autopsy was performed in only 4.3% of patients. Patients who underwent an ESR test had a greater likelihood of being diagnosed with a malignant neoplasm (17.4%) or unknown cause (25.2%) compared to those without an ESR test. Patients who had undergone an imaging examination had a

relatively greater likelihood of being diagnosed with malignancy or NIID compared to those without imaging examinations (**Table 2**).

There was a significant association between the etiology of FUO and the prognosis of patients (**Fig 2**; $\chi 2=27.6$, df=12, p=0.006). Most patients with FUO with different causative diseases generally were cured or experienced relief. However, patients with malignancy or unknown causes had higher mortality rates (22.7% and 12.9%, respectively) (Figure 2). Among all 141 patients, the cause of fever was not identified in 104 patients at 2 months (**Fig 3**). At the end of the follow-up period, the cause of FUO remained unknown in 30 patients and 11 patients were not cured or had no symptom relief. Four deaths occurred among these patients. Pathological autopsy was performed on a small proportion of those who died (n=3); two cases remained unknown after autopsy (**Fig 3**).

Tests were performed for diagnostic evaluation and abnormal readings were defined as in Naito et al.:[1] WBC: 4000-8000; CRP: 0.3; ESR >100 mm/hr and procalcitonin ≥0.25 ng/mL. Most patients with unknown cause of FUO had abnormal WBC and CRP levels (WBC: 56.7%; CRP: 73.3%, respectively) while a smaller percentage of patients had abnormal ESR and procalcitonin levels (ESR: 24.1%; procalcitonin: 23.1%). **Table 3** shows the association of patient demographics, clinical characteristics and diagnostic examinations for patients with known and unknown causes of FUO. There was a

significant association between having undergone ESR examination and unknown final diagnosis of FUO (odds ratio=8.43, 95% confidence interval=1.09-65.00, p=0.041). Furthermore, 80% (28 of 35) of patients with an abnormal ESR value had a final diagnosis. No other variables differed significantly between the groups with known and unknown cause of FUO (all p>0.05) (**Table 3**).

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[a]		Total	Final diagnosis				
			Infection[b]	Malignancy[b]	NIID[b]	Other[b]	Unknown[b]
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 (9.1%)	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[c]	Yes	141	24 (17.0%)	22 (15.6%)	48 (34.0%)	17 (12.1%)	30 (21.3%)
CRP[c]	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.

[a]Missing data would not be reported.

[b]Percentage was calculated as number of patients who performed examination divided by total patients for each condition.

[c]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)	0.566
	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)	0.425
	No	97 (77.6%)	28 (22.4%)	1.00	
Rash	Yes	26 (81.3%)	6 (18.8%)	0.82 (0.30-2.21)	0.692
	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	

[a]Percentage was calculated as the number of patients who received an examination divided by the total patients for each condition.

[b]Chi-square tests were performed.

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

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

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 (≥65 and <65 years), but not with sex. While most studies have identified NIID as the most common cause of FUO in Japan,[1 15 28 29] our 2013 study found similar rates of NIID as a cause of FUO in participants ≥65 and <65 years.[3] 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 ≥65 accounted for 26.7% of the 127.11 million population in 2016,[27 30] and will increase to 40% in 2050, according to a new analysis.[31] In this study, 46.1% of patients were ≥65 years, an increase since 2013 (42.1%).[3] Moreover, this trend should also be considered in Western countries, where aging of the population is also expected.[31] A diagnosis of NIID, which occurs significantly more often in elderly patients,[1] consequently must be considered first for an FUO, particularly in patients ≥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

population depends on the genotype combinations of the HLD DRB1 and DQB1 alleles, and predisposing risk has been found associated with the haplotype DRB1*15:01-DQB1*06:02 in Japanese patients with AOSD.[32] However, genotyping results were not available for this study.

Difference in causative disease between populations could be influenced by factors such as geographic location, zoonotic characteristics and the economic and medical organization of the local health care system. Infectious disease was the leading cause of FUO in South-East Europe, as reported by Baymakova et al. in 2016.[33] Infection was the second the most common causes of fever in our patient population. Our previous study in 2013 demonstrated that PMR and HIV should be considered as causes of FUO.[3] However, HIV was not found in this study, possibly due to the efficiency of HIV testing in Japan. The frequency of unknown cause in our study was comparable to that found previously in 2013.[3]

The availability of new diagnostic techniques, including computed tomography (CT), PET imaging, improved culture techniques and advanced serologic assays has changed both the spectrum of diseases causing FUO and the time to reveal the final diagnosis. In a previous study, the cause of FUO diagnosed after ≥100 days was malignancy.[3] In this study, more than 50% of FUO patients with infections, malignancy, NIID and other causes had a final diagnosis within 100 days of fever onset. Similarly,

in a series of patients with FUO studied in Europe and USA, 30-50% were of unknown cause after a follow-up of ≥100 days.[6 9 34]

In the present study, we evaluated key symptoms and signs in patients with FUO, to determine which were diagnostically useful. We found that comorbidities were the main symptoms and signs in FUO caused by malignant neoplasms. Patients with infectious diseases often had respiratory and gastrointestinal symptoms, while those with NIID often had arthralgia or muscle pain. Although the various symptoms/signs were not directly related to the final diagnosis of FUO,[14] their presence might help improve the differential diagnosis in patients with FUO.

A systemic review from 2003 reported that the prevalence of FUO was 1.5-3% in all hospitalized patients, and mortality in these patients was 12-35%.[35] We found that the etiology of FUO was significantly associated with prognosis; FUO patients diagnosed with malignancy or unknown causes had higher mortality rates. A Danish study also found that FUO patients with malignancy had poor prognosis.[36] Little is known about the prognosis of patients with FUO of unknown cause. In our study, 4 of 30 (13.3%) patients with FUO of unknown cause died during within 6 months; the cause of FUO remained unknown after autopsy in two of these patients. In patients with FUO of unknown cause, Dutch studies showed mortality rates of 2.0-4.0%[6 36] and other western-European studies reported mortality rates of 2.0-19.0%.[7 10 37-39] The variances

among studies may be due to differences in patient selection, study design or health care systems.

Since there is no standard diagnostic approach in FUO, classic test features are difficult to apply in FUO studies. Of all positive biochemical tests, only 1.7% contributed indirectly to diagnosis in a Turkey FUO study.[13] Despite advances in diagnostic tests and techniques, a significant proportion of all cases remains undiagnosed.[40] Our previous study found that 14.9% of FUO patient had an ESR >100 mm/hr, including 5 with FUO of unknown cause[1]. In the current study, 35 of 115 patients (30.4%) had an abnormal ESR test result; in these, the cause of FUO was identified in 80% of patients. In addition, there was a significant association between known cause and ancillary ESR test, but not with other variables such as procalcitonin or PET. Therefore, the current study demonstrated the usefulness of ESR in evaluating FUO. However, further investigation is required. We speculate that future FUO research may be leaving the twilight zone as diagnostic microcellular research technologies emerge from the laboratory to point-of-care rapid diagnostic kits. We await further advances in diagnostic artificial intelligence to expose FUO cause in more cases.[41 42]

The present study has the following limitations. First, despite this being the largest data sample ever collected from geographically-dispersed Japanese hospitals, the

sample size is still small; caution should be taken when generalizing our results. Also, we did not establish uniformity of the diagnostic criterion used in this study, which may have resulted in over- or under-diagnosis of specific disease categories. Uncertainty of diagnosis was not addressed. Finally, our follow-up database was not designed to include records of spontaneous fever remission. [43]

In conclusion, evaluating and determining the cause of a fever is complex. The availability of new diagnostic techniques (including CT and PET imaging), improved culture techniques and recent advances in serologic assays have all changed both the spectrum of diseases causing FUO and the time needed to reach a final diagnosis. Our study identified age and ESR as potentially important factors useful in assisting clinicians navigate the paths to diagnosing FUO. These advances, together with future development of multi-microbial and cancer cell detection tools, may allow faster determination of the causes of FUO and further improve the prognosis of FUO patients.

Funding:

This work was supported by Grants-in-Aid for Scientific Research, Japan Project Number: 16K09257.

Competing interests:

None

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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Acknowledgments:

We would like to acknowledge Convergence CT Japan K.K for conducting statistical

analysis, data interpretation, and reviewing this manuscript. We would also like to acknowledge support from Tatsuo Ishizuka, Kenji Kanazawa, Nobuki Nanki, Megumi Higaki, and Koichi Mashiba for valuable administrative and research assistance throughout this study.

Ethics approval:

Research Ethics Committee of Juntendo University School of Medicine

Data availability statement:

All data generated within this study are available from the corresponding author on request.

REFERENCES

1. Naito T, Torikai K, Mizooka M, et al. Relationships between causes of fever of unknown origin and inflammatory markers: A multicenter collaborative retrospective study *Intern Med*2015;**54**:1989-94.doi:10.2169/internalmedicine.54.3313

2. Cunha BA, Lortholary O, Cunha CB. Fever of unknown origin: a clinical approach. *Am J Med*2015;**128**:1138.doi:10.1016/j.amjmed.2015.06.001

3. Naito T, Mizooka M, Mitsumoto F, et al. Diagnostic workup for fever of unknown origin: a multicenter collaborative retrospective study. *BMJ Open*2013;**3**:e003971.doi:10.1136/bmjopen-2013-003971

4. Alt HL, Barker MH. Fever of unknown origin. *JAMA*1930;**94**:1457–61

5. Petersdorf RG, Beeson PB. Fever of unexplained origin: report on 100 cases. *Medicine (Baltimore)*1961;**40**:1-30

6. de Kleijn EM, Vandenbroucke JP, van der Meer JW. Fever of unknown origin (FUO). I A. prospective multicenter study of 167 patients with FUO, using fixed epidemiologic entry criteria. The Netherlands FUO Study Group. *Medicine (Baltimore)*1997;**76**:392-400

7. Zenone T. Fever of unknown origin in adults: evaluation of 144 cases in a non-university hospital. *Scand J Infect Dis*2006;**38**:632-8. doi:10.1080/00365540600606564

8. Miller RF, Hingorami AD, Foley NM. Pyrexia of undetermined origin in patients with human immunodeficiency virus infection and AIDS. *Int J STD AIDS*1996;**7**:170-5.doi:10.1258/0956462961917564

9. Bleeker-Rovers CP, Vos FJ, de Kleijn EM, et al. A prospective multicenter study on fever of unknown origin: the yield of a structured diagnostic protocol. *Medicine (Baltimore)*2007;**86**:26-38.doi:10.1097/MD.0b013e31802fe858

10. Knockaert DC, Vanderschueren S, Blockmans D. Fever of unknown origin in adults: 40 years on. *J Intern Med*2003;**253**:263-75

11. Durack DT, Street AC. Fever of unknown origin--reexamined and redefined. *Curr Clin Top Infect Dis*1991;**11**:35-51

12. Baymakova M, Popov GT, Andonova R, et al. Fever of unknown origin and Q-

fever: a case series in a Bulgarian hospital. *Caspian J Intern Med*2019;**10**:102-06.doi:10.22088/cjim.10.1.102

13. Kucukardali Y, Oncul O, Cavuslu S, et al. The spectrum of diseases causing fever of unknown origin in Turkey: a multicenter study. *Int J Infect Dis*2008;**12**:71-9.doi:10.1016/j.ijid.2007.04.013

14. Balink H, Verberne HJ, Bennink RJ, et al. A Rationale for the use of F18-FDG PET/CT in fever and inflammation of unknown origin. *Int J Mol Imaging*2012;**2012**:165080.doi:10.1155/2012/165080

15. Takeda R, Mizooka M, Kobayashi T, et al. Key diagnostic features of fever of unknown origin: Medical history and physical findings. *J Gen Fam Med*2017;**18**:131-34.doi:10.1002/jgf2.35

16. Kim YS, Kim KR, Kang JM, et al. Etiology and clinical characteristics of fever of unknown origin in children: a 15-year experience in a single center. *Korean J Pediatr*2017;**60**:77-85.doi:10.3345/kjp.2017.60.3.77

17. Cho CY, Lai CC, Lee ML, et al. Clinical analysis of fever of unknown origin in children: A 10-year experience in a northern Taiwan medical center. *J Microbiol Immunol Infect*2017;**50**:40-45.doi:10.1016/j.jmii.2015.01.001

18. Tal S, Guller V, Gurevich A. Fever of unknown origin in older adults. *Clin Geriatr Med*2007;**23**:649-68, viii.doi:10.1016/j.cger.2007.03.004

19. Simon L, Gauvin F, Amre DK, et al. Serum procalcitonin and C-reactive protein levels as markers of bacterial infection: a systematic review and meta-analysis. *Clin Infect Dis*2004;**39**:206-17.doi:10.1086/421997

20. Kubota K, Nakamoto Y, Tamaki N, et al. FDG-PET for the diagnosis of fever of unknown origin: a Japanese multi-center study. *Ann Nucl Med*2011;**25**:355-64.doi:10.1007/s12149-011-0470-6

21. Kouijzer IJ, Bleeker-Rovers CP, Oyen WJ. FDG-PET in fever of unknown origin. *Semin Nucl Med*2013;**43**:333-9.doi:10.1053/j.semnuclmed.2013.04.005

22. Kouijzer IJE, Mulders-Manders CM, Bleeker-Rovers CP, et al. Fever of unknown origin: the value of FDG-PET/CT. *Semin Nucl Med*2018;**48**:100-07.doi:10.1053/j.semnuclmed.2017.11.004

23. Takeuchi M, Dahabreh IJ, Nihashi T, et al. Nuclear imaging for classic fever of

unknown origin: Meta-analysis. *J Nucl Med*2016;**57**:1913-19.doi:10.2967/jnumed.116.174391

24. Bleeker-Rovers CP. Positron emission tomography with 18F-fluorodeoxyglucose in fever of unknown origin and infectious and non-infectious inflammatory diseases. *Radboud University Nijmegen, Netherlands*2007

25. Unger M, Karanikas G, Kerschbaumer A, et al. Fever of unknown origin (FUO) revised. *Wien Klin Wochenschr*2016;**128**:796-801.doi:10.1007/s00508-016-1083-9

26. Knockaert DC, Vanneste LJ, Bobbaers HJ. Fever of unknown origin in elderly patients. *J Am Geriatr Soc*1993;**41**:1187-92

27. Sudo K, Kobayashi J, Noda S, et al. Japan's healthcare policy for the elderly through the concepts of self-help (Ji-jo), mutual aid (Go-jo), social solidarity care (Kyo-jo), and governmental care (Ko-jo). *Biosci Trends*2018;**12**:7-11.doi:10.5582/bst.2017.01271

28. Iikuni Y, Okada J, Kondo H, et al. Current fever of unknown origin 1982-1992. *Intern Med*1994;**33**:67-73

29. Shoji S, Imamura A, Imai Y, et al. Fever of unknown origin: a review of 80 patients from the Shin'etsu area of Japan from 1986-1992. *Intern Med*1994;**33**:74-6

30. Ren J, Ma R, Zhang ZB, et al. Effects of microRNA-330 on vulnerable atherosclerotic plaques formation and vascular endothelial cell proliferation through the WNT signaling pathway in acute coronary syndrome. *Journal of Cellular Biochemistry*2018;**119**:4514-27.doi:10.1002/jcb.26584

31. He W, Goodkind D, Kowal P. U.S. Census Bureau, International Population Reports, P95/16-1, An Aging World: 2015. Government Publishing Office, Washington, DC. , 2016:1-165.

32. Fujita Y, Furukawa H, Asano T, et al. HLA-DQB1 DPB1 alleles in Japanese patients with adult-onset Still's disease. *Mod Rheumatol*2018:1-5.doi:10.1080/14397595.2018.1514999

33. Baymakova M PK, Dikov I, Popov GT, Mihaylova-Garnizova R, Kovaleva V, Kundurdjiev T. Fever of unknown origin in a Bulgarian hospital: evaluation of 54 cases for a four year-period. *J Clin Anal Med*2016;**7**:70-75.doi:10.4328/JCAM.3897

34. Hersch EC, Oh RC. Prolonged febrile illness and fever of unknown origin in

adults. *Am Fam Physician*2014;**90**:91-6

35. Mourad O, Palda V, Detsky AS. A comprehensive evidence-based approach to fever of unknown origin. *Arch Intern Med*2003;**163**:545-51

36. Mulders-Manders CM, Engwerda C, Simon A, et al. Long-term prognosis, treatment, and outcome of patients with fever of unknown origin in whom no diagnosis was made despite extensive investigation: A questionnaire based study. *Medicine(Baltimore)*2018;**97**:e11241.doi:10.1097/MD.0000000000011241

37. Pedersen TI, Roed C, Knudsen LS, et al. Fever of unknown origin: a retrospective study of 52 cases with evaluation of the diagnostic utility of FDG-PET/CT. *Scand J Infect Dis*2012;**44**:18-23.doi:10.3109/00365548.2011.603741

38. Robine A, Hot A, Maucort-Boulch D, et al. Fever of unknown origin in the 2000s: evaluation of 103 cases over eleven years. *Presse Med*2014;**43**:e233-40.doi:10.1016/j.lpm.2014.02.026

39. Vanderschueren S, Del Biondo E, Ruttens D, et al. Inflammation of unknown origin versus fever of unknown origin: two of a kind. *Eur J Intern Med*2009;**20**:415-8.doi:10.1016/j.ejim.2009.01.002

40. Horowitz HW. Fever of unknown origin or fever of too many origins? *N Engl J Med*2013;**368**:197-9.doi:10.1056/NEJMp1212725

41. Dave VP, Ngo TA, Pernestig AK, et al. MicroRNA amplification and detection technologies: opportunities and challenges for point of care diagnostics. *Lab Invest*2019;**99**:452-69.doi:10.1038/s41374-018-0143-3

42. Liang H, Tsui BY, Ni H, et al. Evaluation and accurate diagnoses of pediatric diseases using artificial intelligence. *Nat Med*2019;**25**:433-38.doi:10.1038/s41591-018-0335-9

43. Takeuchi M, Nihashi T, Gafter-Gvili A, et al. Association of 18F-FDG PET or PET/CT results with spontaneous remission in classic fever of unknown origin: A systematic review and meta-analysis. *Medicine*2018;**97**:43(e12909).doi:10.1097/MD.0000000000012909

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).

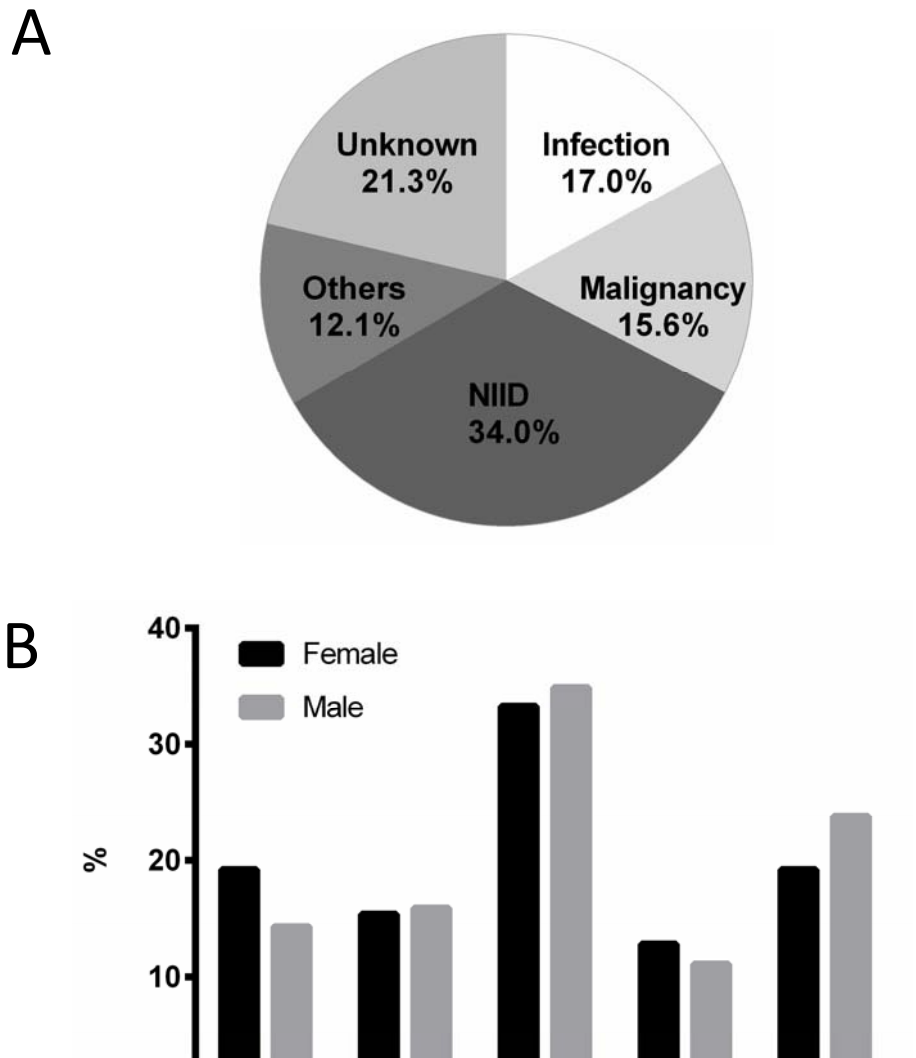
A
Unknown
21.3%
Infection
17.0%
Others
12.1%
Malignancy
15.6%
NIID
34.0%
B
Female
Male
%
40
30
20
10
0
Infection
Malignancy
NIID
Others
Unknown
C
<65 years
≥65 years
%
50
40
30
20
10
0
Infection
Malignancy
NIID
Others
Unknown

Figure 1

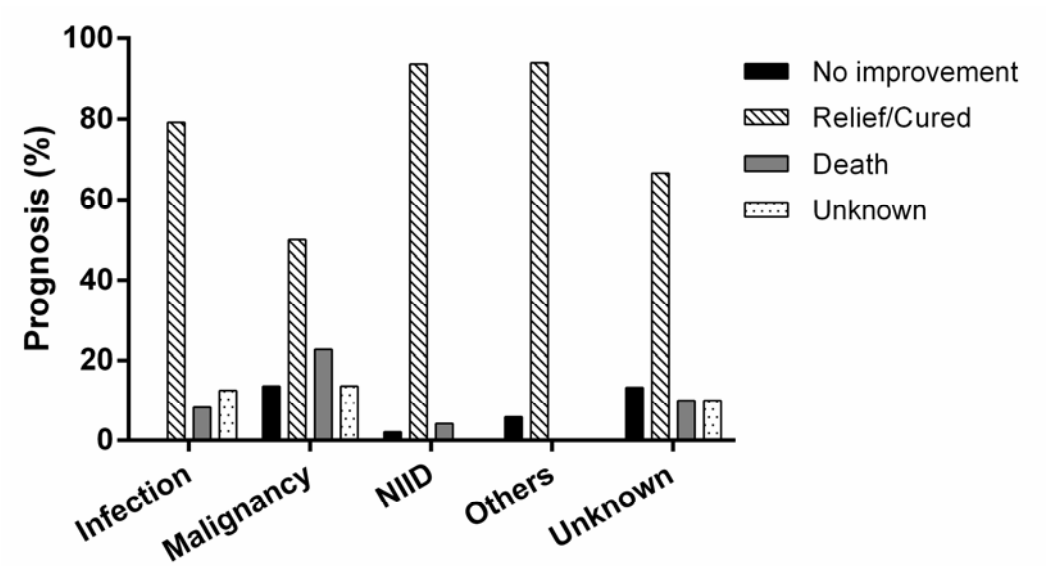
100
80
60
40
20
0
Prognosis (%)
Infection
Malignancy
NIID
Others
Unknown
No improvement
Relief/Cured
Death
Unknown

Figure 2

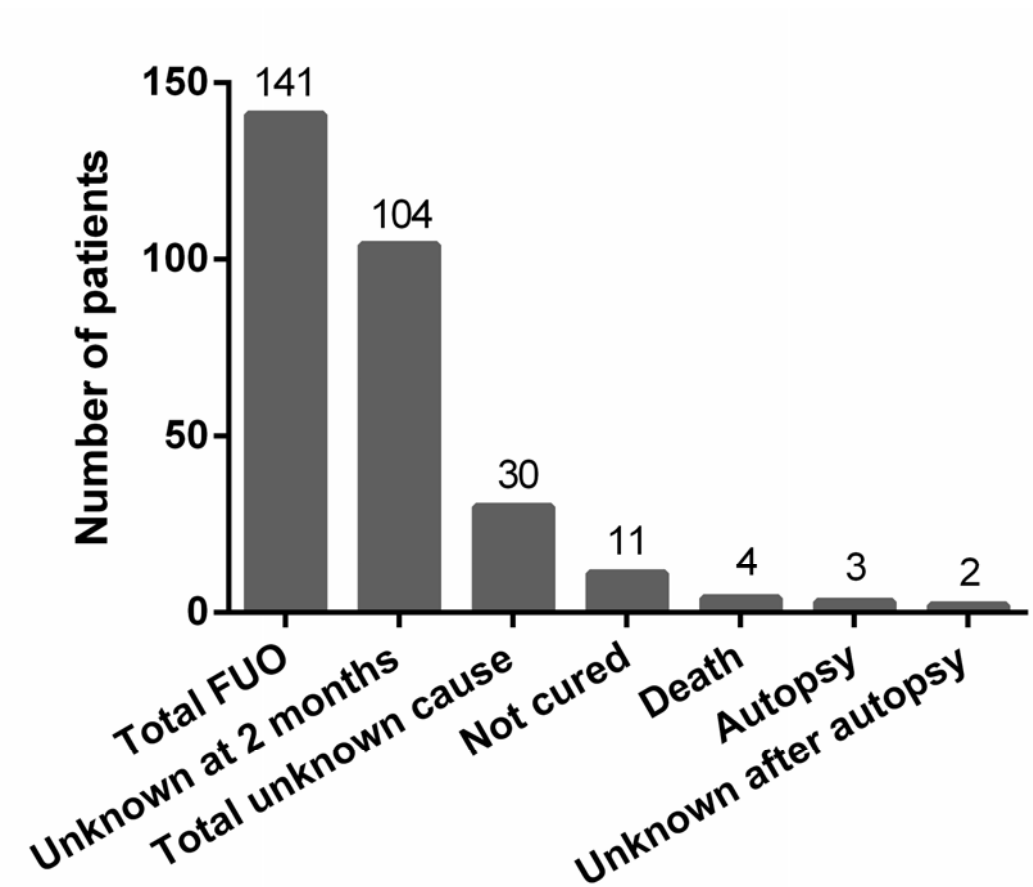
Number of patients
150
100
50
0
141
104
30
11
4
3
2
Total FUO
Unknown at 2 months
Total unknown cause
Not cured
Death
Autopsy
Unknown after autopsy

Figure 3

Supplementary

Supplementary Figure

Figure S1

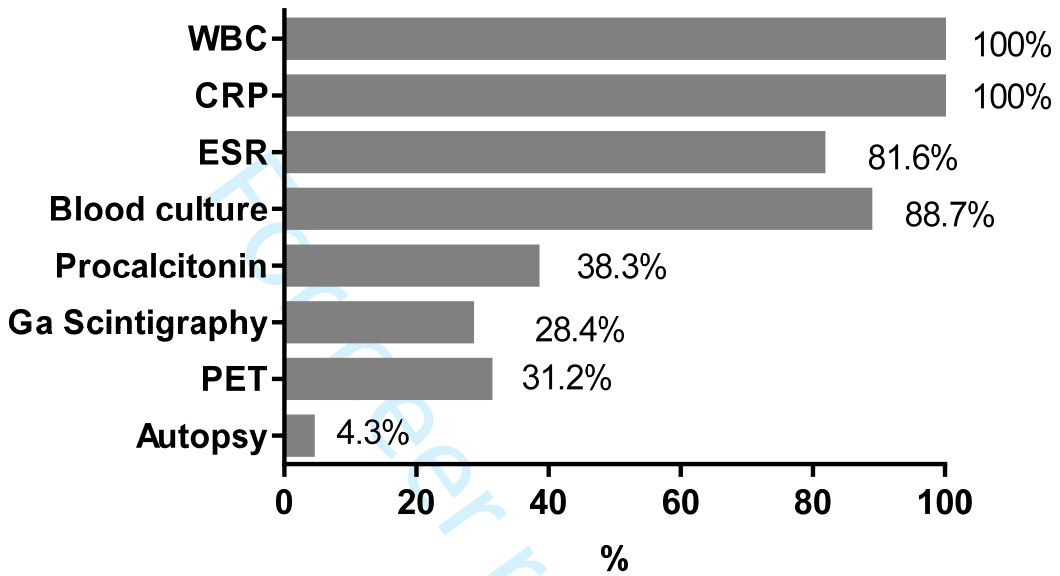

Figure S1. Frequency of examination for diagnostic evaluation. WBC, white blood cells count; CRP, C reactive protein; ESR, erythrocyte sedimentation rate; Ga, gallium; PET, positron emission tomography

Supplementary Tables

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

	Time interval (days)		
Final diagnosis	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
Introduction			
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
Methods			
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*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—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 *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	8
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—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*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	n/a

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	n/a
Results			
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) *Cohort study*—Summarise follow-up time (eg, average and total amount)	9
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	10-11, Figures, sup tables
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	n/a
		Cross-sectional study—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
Discussion			
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
Other information			
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.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE 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 http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.