Journal of Infection and Public Health 18 (2025) 102824

Contents lists available at ScienceDirect

Journal of Infection and Public Health

journal homepage: www.elsevier.com/locate/jiph

Original Article

Epidemiology and diagnostic challenges of fever of unknown origin (FUO) among adults: A multicenter retrospective study in Northern Italy

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ARTICLE INFO

Article history:
Received 9 January 2025
Received in revised form 8 May 2025
Accepted 11 May 2025

Keywords:
Fever of unknown origin
Diagnosis
Antibiotic
Vector borne diseases
Emerging diseases

ABSTRACT

Background: Fever of Unknown Origin (FUO) is broadly defined as a fever with an unidentified cause despite a minimum set of diagnostic investigations. The variability of FUO etiologies across geographic areas, age groups, and decades makes diagnosis challenging and complicates the adoption of a standardized diagnostic approach. Global warming and changing interactions between humans, animals, and the environment are contributing to the emergence and re-emergence of zoonotic infections. Emerging Vector-Borne Disease (VBD) pathogens circulate in Northern Italy, but their prevalence and impact on febrile illnesses remain poorly understood. This study investigated FUO epidemiology, diagnosis, and treatment in three hospitals in Emilia-Romagna (northeastern Italy).

Methods: The medical records from 652 patients who were discharged with the International Classification of Diseases, Ninth Revision (ICD-9) codes "780.6 Fever of unknown origin" and "087.9 Relapsing fever, unspecified" between January 2017 and December 2023 were analysed. Results: Among patients discharged with FUO between 2017 and 2023, the mean age was 58 years, and 45 % older than 65 years. Comorbidities were present in 75 %, and 26 % had active cancer. A diagnostic hypothesis was present in 32 % of cases. Diagnostic tests were mainly laboratory-based; 5.9 % had confirmed infections. Antibiotics were used in 62 % of patients mostly penicillin/beta-lactamase inhibitors. FUO discharges peaked in summer.

Conclusion: Active hospital-based surveillance are crucial to deepen our current understanding on FUO epidemiology and possible contribution of VBD pathogens while refining the use of antibiotics in the clinical practice.

Introduction

Fever of Unknown Origin (FUO) has a relevant impact on healthcare systems, accounting for 2–2.9 % of hospital admissions in previous studies from Italy [1]. FUO was firstly defined in 1961, when Petersdorf and Beeson [2], described as a condition fulfilling the following criteria: 1) Fever lasting at least 3 weeks, 2) Temperature higher than 38.3°C on several occasions, and 3) Uncertain diagnosis after 1 week of in-hospital investigations. The first two criteria allow the exclusion of acute and self-limited viral diseases, such as common viral respiratory tract infections [3], and habitual hyperthermia [4,5]. As of today, FUO remains an enigmatic condition. despite advanced diagnostic techniques [6,7]. Over the last 70 years, the proportion of FUO conditions has risen in industrialized countries [8] with 7–53 % undiagnosed patients [9]. The absence of a universally accepted diagnostic algorithm for the differential

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https://doi.org/10.1016/j.jiph.2025.102824

Abbreviations

AUSL	Azienda Unità Sanitaria Locale
AWaRe	Access, Watch, Reserve
CMV	Cytomegalovirus
COVID-19	Coronavirus Disease of 2019
EBV	Epstein-Barr Virus
FUO	Fever of Unknown Origin
HIV	Human Immunodeficiency Virus
ICD-9	International Classification of Diseases, 9th revision
ID	Infectious Disease
IM	Internal Medicine
M	Median
NIID	Non-infectious Inflammatory Disease
PET/CT	Positron Emission Tomography/Computed Tomography
RTI	Respiratory Tract Infection
SD	Standard Deviation
STI	Sexually Transmitted Disease
TOSV	Toscana Virus
USUV	Usutu Virus
UTI	Urinary Tract Infection
VBD	Vector-borne Disease
WHO	World Health Organization
WNV	West Nile Virus

diagnosis of FUO makes it challenging to identify its causes, often leading to diagnostic failures due to the lack of a standardized protocol [4].

The most common causes of FUO can be grouped into 4 categories: infections, malignancies, non-infectious inflammatory diseases (NIID) and miscellaneous causes [1]. While the number of NIIDs has increased over the past decades[8] particularly in Europe [10–12], infections remain the most frequent cause of FUO on a global scale, accounting for 37 % of cases, though the prevalence and distribution of the infectious agents depend on the region of origin [13]. NIIDs include collagen-vascular diseases (including autoimmune conditions), vasculitis syndromes, and non-infectious granulomatous conditions [13]. Malignancies are the third most common cause of FUO, accounting for 15 % of cases [14], though their prevalence as FUO etiology has decreased due to advancements in imaging techniques [15]. Returning travelers are a subset of FUO patients in which malaria, typhoid fever, and acute HIV infections are commonly recorded. Frequent etiologies are also tuberculosis, visceral leishmaniasis, and hemorrhagic fevers [16]. Thus, travel history, exposure to animals and insects, vector bites, contaminated water and food consumption should be key components of the anamnesis in FUO patients [17]. While vector-borne infections are responsible for 20 % of FUO cases in low-income countries[16], little is known about their prevalence among undiagnosed FUO patients in Europe, and no studies have analyzed seasonal trends in FUO admissions. However, most cases of undiagnosed FUO are self-resolving and could be attributable to a “self-limited prolonged viral infection” [18]. Some viral febrile-neurologic diseases such as *West Nile Virus* (WNV), *Toscana virus* (TOSV), and *Usutu virus* (USUV) are already endemic in Italy. The objective of this study was to investigate the epidemiology, diagnostic strategies and therapeutic approaches, of FUO’s patients through a retrospective analysis of discharges from infectious diseases and internal medicine wards of 3 different hospitals of the Emilia-Romagna region (north-eastern Italy).

Methods

Data collection

Demographic, clinical, and laboratory data were collected from patient medical records during the study period. For each case, the following information was retrieved: age, sex, geographic origin, number of hospital transfers, admission and discharge wards, length of hospital stay, number of admissions (single or multiple for the same diagnosis), discharge diagnosis, mortality, diagnostic workup, and therapeutic protocol. Patients were stratified into two age groups: 18–64 years and ≥ 65 years. Diagnostic investigations included molecular, culture-based, and serological tests aimed at identifying potential infectious etiologies. Specifically, data were gathered on the presence or absence of vector-borne diseases, septic conditions, inflammatory markers, PET/CT imaging, and histopathological examinations. An electronic database was created using Microsoft Excel to archive all demographic and clinical information. The database is stored on the Azienda Unità Sanitaria Locale (AUSL) Romagna institutional server, protected by a password with access restricted to the principal investigator and authorized members of the research team who hold the necessary credentials.

Case definition

A multicentric, retrospective, observational study was conducted by the Microbiology Unit AUSL Romagna. Enrolled patients were aged 18 years or older and had been discharged between January 2017 and December 2023 with the International Classification of Diseases, Ninth Revision (ICD-9) codes “780.6 Fever of unknown origin” and “087.9 Relapsing fever, unspecified” from the Internal Medicine wards of Forlì-Cesena Hospital and the Infectious Diseases wards of Forlì-Cesena, Rimini, and Ravenna Hospitals. Eligible patients had been hospitalized for more than one week with recurrent fever ≥ 38.3°C or for a duration exceeding 21 days. Data on age, sex, place of origin, number of transfers among wards, admission and discharge wards, length of hospitalization, mortality, comorbidities, risk factors, and diagnostic and therapeutic protocols were collected from clinical records of patients.

Inclusion and exclusion criteria

Patients aged over 18 years included in this study were hospitalized for more than one week with recurrent fever ≥ 38.3°C, or with fever lasting more than 21 days. Eligibility required that patients had been informed about the study and had provided written informed consent (Prot. 2219/2024 I.5/199). Following consent, cases were retrospectively identified through the review of medical records. Exclusion criteria included patients under 18 years of age, those diagnosed with FUO in the context of neutropenia or immunosuppressive conditions (e.g., HIV-positive patients or transplant recipients). Pregnant women and patients with nosocomial FUO were also excluded from the study.

Statistical analysis

Descriptive statistics were employed to portray selected variables using frequency distribution, percentage, and frequency tables for qualitative variables and mean, median, standard deviation, and range for quantitative variables. The normality of quantitative variables was assessed using the Shapiro-Wilk test. Depending on the distribution of the data, group comparisons were performed using either parametric tests (*t*-test) or non-parametric tests (Wilcoxon rank-sum test or Kruskal-Wallis test). Differences between qualitative variables were analyzed using Pearson’s *Chi*-squared test (p-value < 0.5). Association plots of standardized residuals were used to

visualize relationships, including only variables with more than 10 cases. All statistical analyses and Figures were performed using R software v. 4.4.1 (R Core Team, 2024. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/). Data manipulation was conducted with the dplyr, tidyr, lubridate, and reshape2 packages; correlation analysis was facilitated by corrplot, enabling intuitive interpretation of variable relationships.

Ethical considerations

The study named FUOR 2024 was approved by the Ethics Committee of Romagna (Comitato Etico della Romagna, CEROM, Italia) under Protocol 2219/2024 I.5/199, and the research was conducted following the ethical guidelines of the 1975 Declaration of Helsinki. Signed informed consents were regularly collected from the patients involved in the study and data sheets were encoded to ensure the anonymity of patient's data.

Results

Demographic characteristics and epidemiology

Of the 716 patients discharged between January 2017 and December 2023 with the ICD-9 codes "780.6 Fever of unknown origin" and "087.9 Relapsing fever, unspecified" and meeting the inclusion criteria, n = 652 provided consent and were thus included in the study.

The age of the study population ranged from 18 to 99 years, with a mean age of 58.0 years (*SD* = 20.2). Overall, 55 % of patients aged between 18 and 64 years old, and 45 % of patients were older than 65 years. The population consisted of 59 % males and 41 % females. Hospitalization lengths are summarized in Table 1.

In total, 75 % of patients had at least one comorbidity, and 26 % of patients had an ongoing active neoplasia. Forlì-Cesena internal medicine wards patients had a higher probability of having comorbidities (92 %, χ^2 (1) = 44.64, $p < .001$) or active cancer (36 %, χ^2 (1) = 10.06, $p = 0.002$) with respect to those from the infectious diseases' wards of the same province. A diagnostic hypothesis was present at discharge in 32 % of patients with no significant differences among age groups and gender was found. FUO discharges were significantly higher during the summer months (May-October) compared to winter months (November-April), both in the infectious diseases' wards ($z = -2.16$, $p = 0.03$) and in the internal medicine wards ($z = -4.09$, $p < 0.001$). The seasonal trends of FUO admissions are illustrated in Fig. 1. Exposure to risk factors (Table 2) was reported in 207 medical records (32 %).

A comparison of all febrile syndromes across the Infectious Diseases wards of the three hospitals (Fig. 2) reveals a decline in the number of diagnosed febrile syndromes during 2020, 2021, and 2022. Notably, the number of FUO cases represents a higher proportion of total discharges compared to other febrile conditions during all the considered timeframes.

Clinical presentation and diagnostic strategies

Constitutional symptoms were the most reported (24 % of patients), followed by gastrointestinal (19 %), respiratory (17 %), neurological (16 %), musculoskeletal (14 %), dermatological (10 %), other symptoms (9 %), urinary (8 %), and ophthalmological symptoms (1 %). With regards to clinical presentation, no additional complaints besides fever were reported by 32 % of patients. Due to the routine screening during the COVID-19 pandemic, the use of molecular tests significantly increased, rising from 19 % in 2019 to 79 % in 2020 and 2021, 63 % in 2022, and 50 % in 2023. To reduce potential bias, COVID-19 molecular tests were excluded from the overall analysis. Among all diagnostic tests, laboratory tests including hematological tests, biochemical tests, and urinalysis were the most common (49 % of all diagnostic tests), followed by microbiological serology (18 %), imaging (14 %), and microbiological culture (9 %). Viral aetiology was the most common diagnostic hypothesis, suspected in 8.0 % of patients. The second most frequent diagnostic hypothesis was urinary tract infection (UTI), in 5.5 % of cases, followed by respiratory tract infections (2.6 %), neoplastic or paraneoplastic etiology (2.5 %), pericarditis or endocarditis (1.6 %), and rheumatologic disease (1.5 %). Overall, 39 out of the 652 patients (5.9 %) had at least one positive test indicating an active infection. Of the positive tests, 32/652 (5.1 %) were bacterial infections, 8/652 (1.2 %) were viral infections, and 1 was a parasitic infection. The most frequent positive result was from urine cultures (n = 13). Of those, 2 were positive for *E. coli*, one for *P. mirabilis*, one for *E. faecium*, one for *E. faecalis*, and one for *K. pneumoniae*; the remaining 7 were not specified. In five cases, urinary tract infection was the proposed diagnosis; in others, no hypothesis was recorded, or fever wasn't linked to urinary bacteria. Fig. 3 shows the pathogen testing frequency across wards. Positive associations were found between recent travel and tests for malaria, arbovirus, and STIs. Contact with animals, rural living, and arthropod bites were negatively linked to arbovirus testing but positively associated with zoonotic bacteria tests. Insect bites correlated positively with parasite testing (Fig. 4).

Treatment

Table 3 shows the drug classes used to treat FUO patients. Among those who received antibiotics, 34 % were cancer patients, in fact a

Table 1
Number of patients meeting FUO criteria and definitions.

Characteristic	Overall N = 652	IM Forlì-Cesena N = 146	ID Forlì-Cesena N = 82	ID Ravenna N = 129	ID Rimini N = 295
Days of hospitalization, mean ± SD	6.6 ± 4.8	7.6 ± 5.4	6.3 ± 4.2	8.1 ± 6.3	5.6 ± 3.4
Fever duration, n (%)					
< 3 weeks	300 (75)	42 (71)	31 (62)	64 (74)	163 (80)
≥ 3 weeks	101 (25)	17 (29)	19 (38)	23 (26)	42 (20)
Unknown	251	87	32	42	90
Peak temperature, n (%)					
< 38.3°C	96 (37)	19 (40)	9 (23)	22 (39)	46 (40)
≥ 38.3°C	165 (63)	29 (60)	31 (78)	35 (61)	70 (60)
Unknown	391	98	42	72	179
Petersdorf-Beeson criteria, n (%)	21 (8.0)	4 (8.3)	6 (15)	6 (11)	5 (4.3)
Unknown	391	98	42	72	179
Durack-Street criteria, n (%)	37 (14)	6 (13)	12 (30)	7 (12)	12 (10)
Unknown	391	98	42	72	179

IM = Internal Medicine; ID = Infectious Diseases.

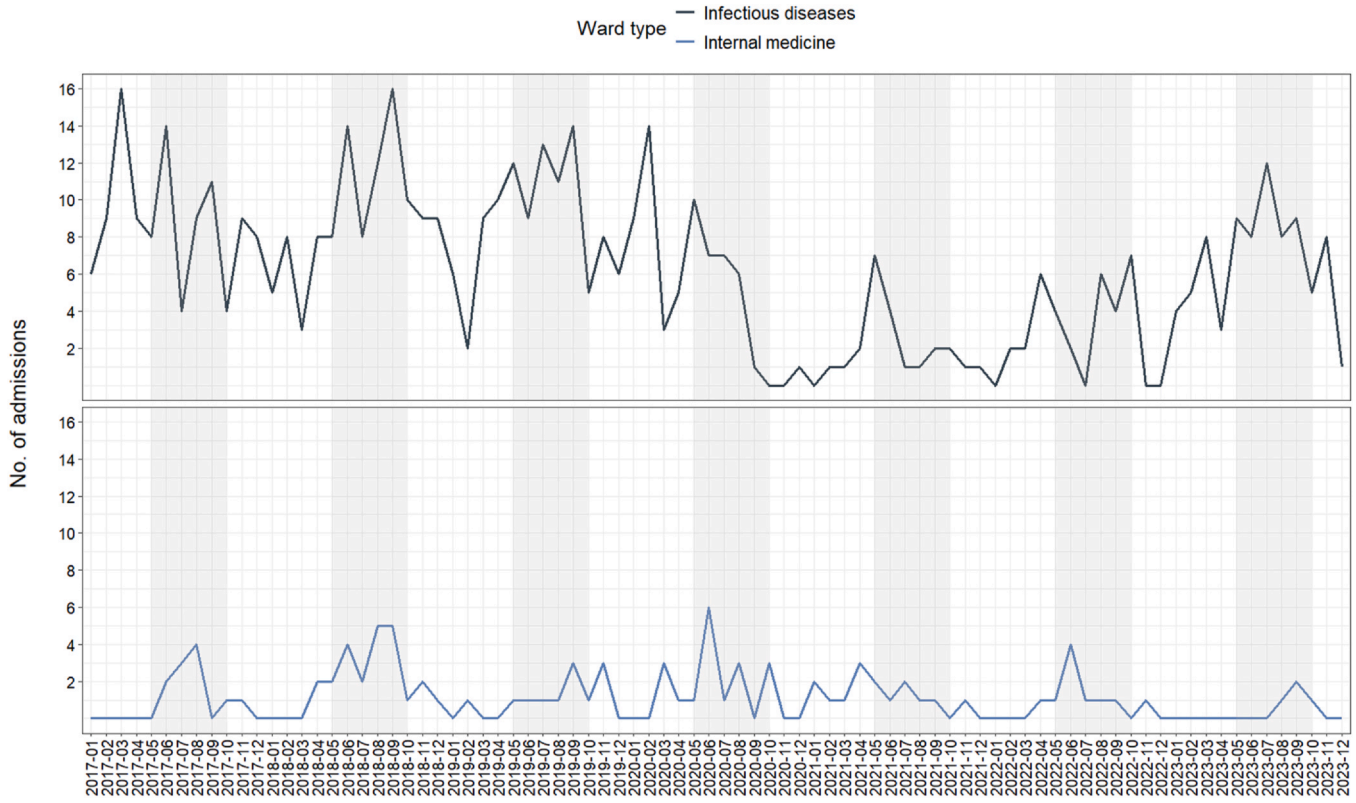

Fig. 1. Seasonal trends of FUO admissions. The trend for the Infectious Diseases ward is shown in charcoal gray, while the Internal Medicine ward is represented in glaucous gray. The summer periods are highlighted in light gray.

Table 2
Exposure history and seasonal distribution of risk factors in FUO patients.

Exposure history	N = 652	Summer N = 390[a]	Winter N = 262[a]
Travel, environmental, and animal exposure, n (%)			
Recent travels abroad	37 (5.7)	21 (57)	16 (43)
Contact with animals/lives in rural area	15 (2.3)	9 (60)	6 (40)
Arthropod bite/contact	14 (2.1)	12 (86)	2 (14)
Recent outdoors activities	4 (0.6)	3 (75)	1 (25)
Flood responder	1 (0.2)	1 (100)	0 (0)
Raw fish consumption	1 (0.2)	1 (100)	0 (0)
Occupational exposure, n (%)			
Works in meat industry	1 (0.2)	1 (100)	0 (0)
Works in port area	1 (0.2)	1 (100)	0 (0)
Medical history, procedures, and device use, n (%)			
Chemotherapy	52 (8.0)	35 (67)	17 (33)
Febrile neutropenia	35 (5.4)	23 (66)	12 (34)
Recent surgery or other invasive procedures	25 (3.8)	14 (56)	11 (44)
Recent infection or hospitalization	19 (2.9)	14 (74)	5 (26)
Others	21 (3.2)	14 (67)	7 (33)
Behavioral and social exposure, n (%)			
Unprotected sex	1 (0.2)	1 (100)	0 (0)
EV substance use	1 (0.2)	0 (0)	1 (100)

[a] Row-relative frequency.

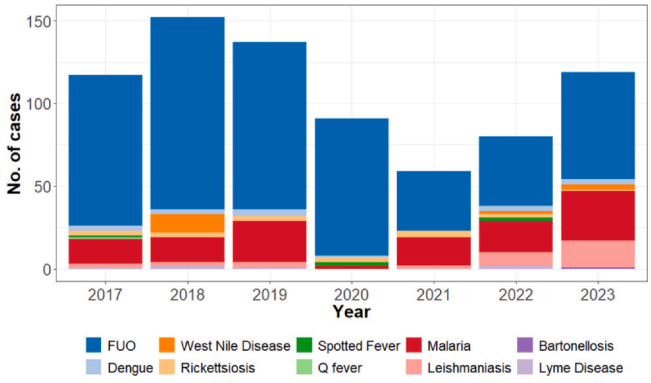

Fig. 2. Comparison of febrile neurological syndromes. Diagnoses from 2017 to 2023 in the Infectious Diseases wards of the hospitals included in this study (FC, RI, RA).

significant association was found between being a cancer patient and receiving antibiotics (χ^2 (1) = 36.08, $p < 0.001$).

The duration of hospitalization in patients who received antibiotics (*M (days)* = 7.3, *SD* = 4.9) was higher than that of patients who didn't (*M* (days) = 5.5, *SD* = 4.4). Wilcoxon rank sum test revealed a significant difference in mean days of hospitalization between age groups ($z = -3.14$, $p = 0.002$), with older patients more likely to be hospitalized for longer periods. A significant relationship was found between age and antibiotic administration (χ^2 (1) = 13.75, $p < 0.001$).

Patients aged 65 or older are more likely to receive antibiotics (70 %) compared to younger patients (55 %). The most frequently used antimicrobial class was penicillin/beta-lactamase inhibitors, which were administered to 33 % of patients. This is followed by third-generation cephalosporins, used in 19 % of patients, and fluoroquinolones, used in 7.5 % of patients. UTI was positively associated to third-generation cephalosporins (37 %) and sulfonamides (5.6 %). Respiratory tract infections (RTIs) were also associated with multiple antibiotics, but the strongest relationship was found with macrolides. Among the other hypotheses, the strongest associations were found between neoplastic or paraneoplastic origin, carbapenems, and penicillin-beta lactamase inhibitors, as well as rheumatologic diseases and penicillin-beta lactamase inhibitors, among others. Overall, 67 % of patients who received an antibiotic did not have a diagnostic hypothesis.

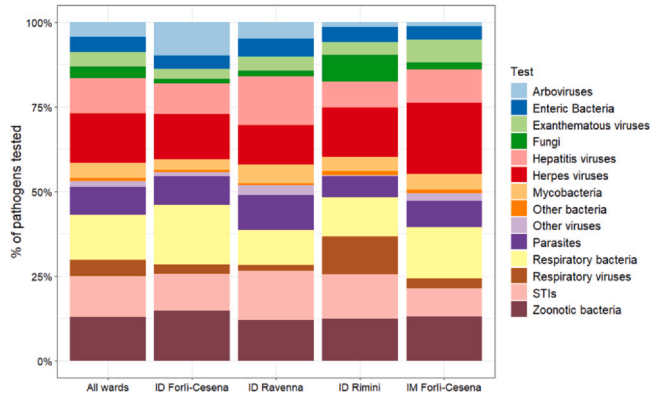

Fig. 3. Relative frequencies of pathogens tested in FUO patients. Tests have been categorized as: arboviruses, enteric bacteria, exanthemous viruses, fungi, *hepatitis viruses, herpes viruses, mycobacteria*, other bacteria (e.g., *S. pyogenes*, group A. streptococci), other viruses (e.g., *Adenovirus, Enterovirus*), parasites (e.g., *T. gondii, Leishmania spp., Strongyloides spp., E. histolytica, Echinococcus spp., Schistosoma spp.* malaria plasmodia and stool parasites), respiratory bacteria, respiratory viruses, agents of sexually transmitted diseases (STIs) and zoonotic bacteria (e.g., *Bartonella spp., Brucella spp., R. rickettsii/typhi, F. tularensis, C. burnetii, Leptospira spp., Borrelia spp.*). ID = Infectious Disease ward; IM = Internal Medicine ward.

Discussion

This study demonstrated that most patients discharged as "Fever of unknown Origin" were not compatible with previous definitions, in fact 75 % of cases the duration of fever was lower than 3 weeks with a median duration of 9 days. The one week of hospitalization, required to meet Petersdorf and Beeson's criteria, reflects the time needed in the '60 s to complete basic investigations [19]. In high-income countries, rapid diagnostic testing (a few hours to days) and the short duration of most fevers likely explain the low number of cases matching the 1967 criteria. A 2022 review [20] suggested that although FUO definitions can be debated, its primary characteristics include an unidentified fever etiology despite conducting a minimum set of investigations, and a sufficiently prolonged fever duration to exclude self-limiting causes. The shorter duration of fever observed in most patients in our cohort suggests that some cases may be attributable to acute infections.

The mean age of FUO patients was 58 years, with 55 % of patients in the 18–64 age group. This aligns with findings from a similar study by Nicolotti et al. [4], where 55 % of patients were in the same age category. The average duration of hospitalization was higher in elderly (≥65 years old) at 7.2 days compared to younger patients (18–64 years old) at 6.1 days, which also reflects the findings previous findings [4]. Average hospitalization days were highest in patients treated with antibiotics, with elderly patients more likely to receive antibiotics. These findings are likely to be interrelated, as in elderly patients with FUO and deteriorating health conditions, a cycle of 10–14 days of broad-spectrum antibiotics is recommended after diagnostic studies have been completed [7]. As expected, the average age and the proportion of patients with comorbidities were higher in the internal medicine ward, whose patients are typically older and multimorbid [21]. Of the patients treated with antibiotics, 34 % were diagnosed with cancer and 8 % had febrile neutropenia. This is consistent with guidelines recommending immediate empiric antibiotic treatment for febrile neutropenic patients [22,23]. Because 70 % of febrile neutropenia episodes respond to the initial empirical antibiotics, most of the fever episodes in neutropenic patients are classified as FUO as their infectious etiology remains unknown [24].

Antibiotics were used in 62 % of patients and the most used molecules were penicillin/beta-lactamase inhibitors (33 % of patients) and third-generation cephalosporins (19 % of patients). The use of antibiotics in our cohort seems lower than what reported from the literature of most low and medium-income countries [6,25–28] but higher than that reported in European studies [1,4,5,9,12,16]. Our data showed that 8 % of antibiotics were administered also in cases where a non-infectious etiology was suspected, and in 48 % of patients in which FUO was attributed to a possible viral infection with a positive association with third-generation cephalosporins and tetracyclines.

According to the AWaRe (Access, Watch, Reserve) classification introduced by the WHO in 2017, most third-generation cephalosporins are categorized as 'Watch' antibiotics, which have a higher risk of promoting resistance and should be reserved for specific, limited infectious syndromes [29]. However, their use in this cohort was widespread and, in most cases, not supported by microbiological evidence.

Blood cultures were performed in 74 % of patients and urine cultures in 58 %. This is consistent with other studies [30], where blood cultures were performed on 86.6 % of patients. Although blood cultures are essential for detecting bacterial infections, only 3 cases were positive in this study. This aligns with previous findings [31], suggesting that cultures should be based on potential diagnostic

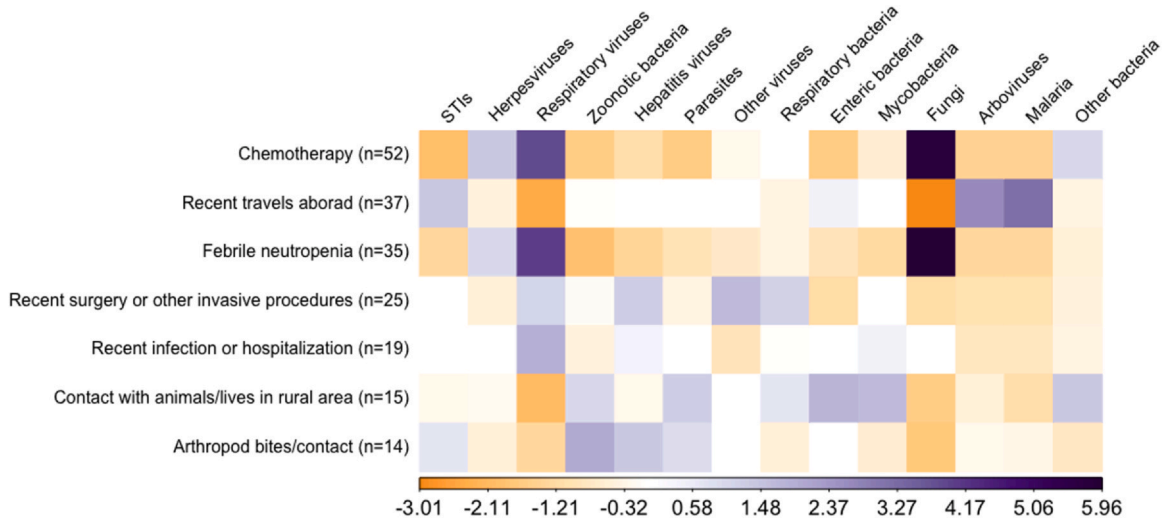

Fig. 4. Risk factors and pathogen associations. Association plot showing Pearson's *chi*-squared test residuals between common risk factors and pathogens ($\chi^2(78) = 225.38$, $p < .001$). Positive residuals (purple) indicate a positive association, while negative residuals (yellow) indicate a negative association.

Table 3
Drug classes used to treat FUO patients.

Drug class, n (%)	Overall N = 652	IM Forlì-Cesena N = 146	ID Forlì-Cesena N = 82	ID Ravenna N = 129	ID Rimini N = 295
Antibiotics	401 (62)	109 (75)	49 (60)	74 (57)	169 (57)
Paracetamol	276 (42)	86 (59)	61 (74)	52 (40)	77 (26)
Glucocorticoid	103 (16)	17 (12)	10 (12)	14 (11)	62 (21)
NSAIDs	48 (7.4)	10 (6.8)	13 (16)	12 (9.3)	13 (4.4)
Opioids	24 (3.7)	4 (2.7)	6 (7.3)	4 (3.1)	10 (3.4)
Antiviral therapy	23 (3.5)	1 (0.7)	4 (4.9)	9 (7.0)	9 (3.1)
Combined analgesics	12 (1.8)	0 (0)	6 (7.3)	0 (0)	6 (2.0)
Other drug class	14 (2.1)	2 (1.4)	2 (2.4)	3 (2.3)	7 (2.4)
Antihistamine	7 (1.1)	1 (0.7)	0 (0)	3 (2.3)	3 (1.0)
Anti gout	5 (0.8)	1 (0.7)	2 (2.4)	0 (0)	2 (0.7)
Anticholinergic	1 (0.2)	0 (0)	0 (0)	0 (0)	1 (0.3)
Antiprotozoal	1 (0.2)	0 (0)	0 (0)	0 (0)	1 (0.3)

IM = Internal Medicine; ID = Infectious Diseases.

clues rather than used as a screening practice in FUO patients. Among the diagnostic hypotheses, the most common are viral infection, urinary tract infection, respiratory tract infection, and fever of neoplastic or paraneoplastic etiology.

Most tested pathogens were herpes viruses (mainly CMV and EBV), zoonotic bacteria (*Borrelia and Brucella spp.*), and respiratory bacteria. Arbovirus testing was conducted in 4.6 % of patients. The infectious nature of most hypotheses aligns with the fact that infections are still the main cause of FUO on a global scale [32].

Among patients with suspected viral etiology, the majority (40 %) were discharged from the internal medicine wards of Forlì-Cesena, the province with the highest incidence of arboviral infections. Another 35 % of these patients were discharged from Rimini, a hospital where fewer patients were tested or diagnosed with arboviral infections, possibly suggesting that testing for arboviruses may not be commonly practiced in the absence of associated neurological symptoms. The number of patients discharged in the summer months was found to be significantly higher than that of admitted in the winter months. Fig. 2 evidenced a decline in patients diagnosed with FUOs from 2020 to 2022, this may align with the COVID-19 pandemic time when diagnostic efforts were primarily targeted to this disease leading to a potential underestimation of other conditions. The similarity of COVID-19 with some febrile syndromes, combined with the pressure on healthcare systems, may have affected accurate diagnosis and management [33,34]. To the Authors' knowledge, there are no other studies that assessed the seasonal trend of FUO cases, except for an Armenian study evaluating the seasonal pattern of FUO attributed to brucellosis [27]. Summer months correspond to the peak activity period of arthropods such as mosquitoes and sandflies and our data may highlight the possible correlation between FUO and Vector-Borne Diseases. The clinical symptoms of VBDs are typically categorized into four primary syndromes: isolated fever or fever accompanied by rash and arthralgia, neurological symptoms, and/or hemorrhagic symptoms [35]. The arboviruses such as *West Nile virus, Usutu virus* and *Toscana virus*, known to be circulating in the study area, most often present with fever, headaches, rash, arthromyalgia, and gastrointestinal disturbances[36]. In our patient cohort, the majority reported fever without additional symptoms. When patients present symptoms of encephalitis or meningitis, arboviruses and other vector-borne diseases, such as leishmaniasis or tick-borne fever, are likely to be tested, as they are also found in the area, thus nowadays established in its differential diagnosis [37]. On the other hand, when patients present with isolated fever or fever with mild symptoms, which is the most common presentation of patients in this study, their testing is probably less likely to take place. Given that VBDs circulation in the study areas is supported by a consistent number of diagnoses, particularly during the peak months of FUO admissions, it is plausible that some of the self-limiting febrile illnesses were also caused by arboviruses and other VBDs, which were not tested due to their mild presentation and lack of severe neurological disease. In fact, between 2017 and 2023, 277 patients were discharged from hospitals of Romagna with confirmed vector-borne diseases. Of these, 191 cases occurred between May and October (Fig. 2). Overall, only 11 % of the medical records reported risk factors (Table 2). A total of 5.7 % of patients reported recent travel abroad, with travelers constituting 50 % of those tested for arboviral infections. Guidelines recommend inquiring about exposure to unclean water, unpasteurized dairy, insect and animal bites, and specific activities when managing fever in returning travelers. Due to climate changes, formerly defined tropical arboviral diseases are increasingly likely to occur in Europe, suggesting that these risk factors should also be assessed in patients residing in endemic areas. Beyond recent travel abroad, potential risk factors for arboviral infections, such as animal contact, rural living, and arthropod bites, are rarely addressing arboviral testing despite being relevant. Evaluating these risk factors might guide targeted testing thereby facilitating the diagnostic process to avoid unnecessary diagnostics and guide the therapeutic approach. Entomological surveillance carried out in the study area detected lesser-known arboviruses, including *Tahyna* and *Fermo viruses* [38,39]. *Fermo virus*'s pathogenicity in humans remains unclear, but it's found in domestic animals in Northern Italy. *Tahyna virus*, widespread in Europe, can mildly affect health but has been linked to central nervous system impairment. These viruses are not yet part of current diagnostic panels, making it difficult to gauge their true impact in patients.

This retrospective study examined FUO cases across four wards, revealing many patients did not meet traditional FUO criteria, with fevers often lasting less than three weeks. Increased FUO cases in summer, and short fever durations lead to suspect unspecified viral infections not yet included in the routine diagnostic panels. For more than 20 years, the Emilia-Romagna region has adopted a one-health approach to the surveillance of some VBDs that became endemic. The active West Nile disease surveillance led to the identification of *Orthobunyavirus* and *Phlebovirus* species and the integrated *Leishmania* surveillance allowed the identification of several arboviruses that may play a role in some of the identified FUOs. Our results suggest the need to integrate the vector-borne diagnostic panels and confirm the importance of syndromic surveillance as an early warning system. This proactive approach could bridge existing gaps in surveillance, ensure better patients' outcomes, and guide effective public health responses to emerging arboviral threats while fighting antibiotic resistance.

Limitations of the study

This study has some limitations, primarily related to its retrospective design. Data were obtained from medical records, which in some cases were incomplete or lacked essential clinical information, potentially leading to gaps in patient histories. Furthermore,

obtaining informed consent from all eligible participants was challenging due to organizational constraints that affected the timely coordination of consent procedures. The inclusion of cancer patients may also introduce bias, as these individuals are often immunocompromised and follow distinct clinical pathways, which could influence the presentation, diagnostic approach, and management of fever of unknown origin compared to the general population.

CRediT authorship contribution statement

Alessandra Mistral De Pascali: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft; **Ludovica Ingletto:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft; **Arianna Succi:** Conceptualization, Data curation, Formal analysis, Investigation, Writing – original draft; **Martina Brandolini:** Conceptualization, Data curation, Investigation; **Laura Dionisi:** Formal analysis; **Claudia Colosimo:** Formal analysis; **Giulia Gatti:** Formal analysis; **Giorgio Dirani:** Supervision; **Silvia Zannoli:** Supervision; **Valeria Frassineti:** Conceptualization, Project administration, Resources; **Giulia Silvestrini:** Conceptualization, Project administration, Resources, **Carlo Biagetti:** Resources; **Francesco Cristini:** Resources; **Paolo Bassi:** Resources; **Monica Cricca:** Validation, Writing – review & editing; **Vittorio Sambri:** Conceptualization, Funding acquisition, Project administration, Validation, Writing – review & editing; **Alessandra Scagliarini:** Conceptualization, Data curation, Methodology, Validation, Writing – original draft; Writing – review & editing.

Funding source

This work was supported by EU funding within the MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases (Project no. PE00000007, INF-ACT).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Authors would like to acknowledge the Local Health Authority of Romagna for collaboration and support.

References

[1] Mansueto P, Di Lorenzo G, Rizzo M, Di Rosa S, Vitale G, Rini G, et al. Fever of unknown origin in a Mediterranean survey from a division of internal medicine: report of 91 cases during a 12-year-period (1991-2002). Intern Emerg Med 2008. https://doi.org/10.1007/s11739-008-0129-z
[2] Petersdorf RG, Beeson PB. Fever of unexplained origin: report on 100 cases. Med (U S) 1961. https://doi.org/10.1097/00005792-196102000-00001
[3] DiNubile MJ. Acute fevers of unknown origin: a plea for restraint. Arch Intern Med 1993. https://doi.org/10.1001/archinte.1993.00410220021002
[4] Nicolotti N, Cattel C, Gualano MR, Soriano A, Manna R, Boccia S. A retrospective analysis of 3 156 admissions with fever of unknown origin in a large Italian hospital. Epidemiol Biostat Public Health 2022;10. https://doi.org/10.2427/8776
[5] Fernandes e Santana L, de Sousa Rodrigues M, de Araújo Silva MP, de Brito RJVC, Nicacio JM, de Castro Duarte RMS, et al. Fever of unknown origin – a literature review. Rev Assoc Med Bras 2019. https://doi.org/10.1590/1806-9282.65.8.1109
[6] Shang J, Yan L, Du L, Liang L, Zhou Q, Liang T, et al. Recent trends in the distribution of causative diseases of fever of unknown origin. Wien Klin Woche 2017. https://doi.org/10.1007/s00508-016-1159-6
[7] Tal S, Guller V, Gurevich A. Fever of unknown origin in older adults. Clin Geriatr Med 2007;23:649–68. https://doi.org/10.1016/j.cger.2007.03.004
[8] Wright WF, Stelmash L, Betrains A, Mulders-Manders CM, Rovers CP, Vanderschueren S, et al. Recommendations for updating fever and inflammation of unknown origin from a modified Delphi Consensus Panel. Open Forum Infect Dis 2024;11:ofae298. https://doi.org/10.1093/ofid/ofae298
[9] Mulders-Manders C, Simon A, Bleeker-Rovers C. Fever of unknown origin. Clin Med, J R Coll Physicians Lond 2015. https://doi.org/10.7861/clinmedicine.15-3-280
[10] Bleeker-Rovers CP, Vos FJ, De Kleijn EMHA, Mudde AH, Dofferhoff TSM, Richter C, et al. A prospective multicenter study on fever of unknown origin: the yield of a structured diagnostic protocol. Medicine 2007. https://doi.org/10.1097/MD.0b013e31802fe858
[11] Torné Cachot J, Baucells Azcona JM, Blanch Falp J, Camell Ilari H. Classic fever of unknown origin: analysis of a cohort of 87 patients according to the definition with qualitative study criterion. Med Clin (Barc) 2021. https://doi.org/10.1016/j.medcli.2020.03.014
[12] Robine A, Hot A, Maucort-Boulch D, Iwaz J, Broussolle C, Sève P. Fever of unknown origin in the 2000s: evaluation of 103 cases over eleven years. Presse Med 2014;43:e233–40. https://doi.org/10.1016/j.lpm.2014.02.026
[13] Wright WF, Yenokyan G, Simner PJ, Carroll KC, Auwaerter PG. Geographic variation of infectious disease diagnoses among patients with fever of unknown origin: a systematic review and meta-analysis. Open Forum Infect Dis 2022. https://doi.org/10.1093/ofid/ofac151
[14] Loizidou A, Aoun M, Klastersky J. Fever of unknown origin in cancer patients. Crit Rev Oncol Hematol 2016;101:125–30. https://doi.org/10.1016/j.critrevonc.2016.02.015
[15] Mourad O, Palda V, Detsky AS. A comprehensive evidence-based approach to fever of unknown origin. Arch Intern Med 2003. https://doi.org/10.1001/archinte.163.5.545
[16] Erdem H, Baymakova M, Alkan S, Letaief A, Yahia WBen, Dayyab F, et al. Classical fever of unknown origin in 21 countries with different economic development: an international ID-IRI study. Eur J Clin Microbiol Infect Dis 2023. https://doi.org/10.1007/s10096-023-04561-5
[17] Botelho-Nevers E, Raoult D. Fever of unknown origin due to rickettsioses. Infect Dis Clin North Am 2007;21:997–1011. https://doi.org/10.1016/j.idc.2007.08.002
[18] Bryan CS, Ahuja D. Fever of unknown origin: is there a role for empiric therapy? Infect Dis Clin North Am 2007;21:1213–20. https://doi.org/10.1016/j.idc.2007.08.007
[19] De Kleijn EMHA, Knockaert DC, Van der Meer JWM. Fever of unknown origin: a new definition and proposal for diagnostic work-up. Eur J Intern Med 2000. https://doi.org/10.1016/S0953-6205(99)00073-4
[20] Haidar G, Singh N. Fever of unknown origin. N Engl J Med 2022;386:463–77. https://doi.org/10.1056/NEJMra2111003
[21] Buurman BM, Frenkel WJ, Abu-Hanna A, Parlevliet JL, De Rooij SE. Acute and chronic diseases as part of multimorbidity in acutely hospitalized older patients. Eur J Intern Med 2016;27:68–75. https://doi.org/10.1016/j.ejim.2015.09.021
[22] Flowers CR, Seidenfeld J, Bow EJ, Karten C, Gleason C, Hawley DK, et al. Antimicrobial prophylaxis and outpatient management of fever and neutropenia in adults treated for malignancy: American society of clinical oncology clinical practice guideline. J Clin Oncol 2013. https://doi.org/10.1200/JCO.2012.45.8661
[23] Lucas AJ, Olin JL, Coleman MD. Management and preventive measures for febrile neutropenia. P T 2018.
[24] Antoniadou A, Giamarellou H. Fever of unknown origin in febrile leukopenia. Infect Dis Clin North Am 2007;21:1055–90. https://doi.org/10.1016/j.idc.2007.08.008
[25] Kabapy AF, Kotkat AM, Shatat HZ. El-Wahab EWA. Clinico-epidemiological profile of fever of unknown origin in an Egyptian setting: a hospital-based study (2009–2010). J Infect Dev Ctries 2016. https://doi.org/10.3855/jidc.7198
[26] Liu C, Liu X, Shi X. Clinical features of spontaneous remission in the classic fever of unknown origin: a retrospective study. Chin Med Sci J 2022. https://doi.org/10.24920/003975
[27] Paronyan L, Zardaryan E, Bakunts V, Gevorgyan Z, Asoyan V, Apresyan H, et al. A retrospective chart review study to describe selected zoonotic and arboviral etiologies in hospitalized febrile patients in the Republic of Armenia. BMC Infect Dis 2016. https://doi.org/10.1186/s12879-016-1764-z
[28] Tan Y, Liu X, Shi X. Clinical features and outcomes of patients with fever of unknown origin: a retrospective study. BMC Infect Dis 2019. https://doi.org/10.1186/s12879-019-3834-5
[29] Zanichelli V, Sharland M, Cappello B, Moja L, Getahun H, Pessoa-Silva C, et al. The WHO AWaRe (Access, Watch, Reserve) antibiotic book and prevention of antimicrobial resistance. Bull World Health Organ 2023. https://doi.org/10.2471/BLT.22.288614
[30] Naito T, Mizooka M, Mitsumoto F, Kanazawa K, Torikai K, Ohno S, et al. Diagnostic workup for fever of unknown origin: a multicenter collaborative retrospective study. BMJ Open 2013;3:e003971. https://doi.org/10.1136/bmjopen-2013-003971
[31] De Kleijn EMHA, Van Lier HJJ, Van Der Meer JWM. Fever of unknown origin (FUO): II. Diagnostic procedures in a prospective multicenter study of 167 patients. Medicine 1997. https://doi.org/10.1097/00005792-199711000-00003
[32] Fusco FM, Pisapia R, Nardiello S, Cicala SD, Gaeta GB, Brancaccio G. Fever of unknown origin (FUO): which are the factors influencing the final diagnosis? A 2005–2015 systematic review. BMC Infect Dis 2019;19:653. https://doi.org/10.1186/s12879-019-4285-8

[33] Prapty CNBS, Ahmed N, Araf Y, Yang Z, Zhai J, Hosen MJ, et al. Coinfection of COVID-19 and dengue: a case report. Front Med (Lausanne) 2022. https://doi.org/10.3389/fmed.2022.872627
[34] Sebastião CS, Gaston C, Paixão JP, Sacomboio ENM, Neto Z, de Vasconcelos JN, et al. Coinfection between SARS-CoV-2 and vector-borne diseases in Luanda, Angola. J Med Virol 2022. https://doi.org/10.1002/jmv.27354
[35] Eckerle, Briciu I, Ergönül VT, Lupşe M, Papa A, Radulescu A, et al. Emerging souvenirs—clinical presentation of the returning traveller with imported arbovirus infections in Europe. Clin Microbiol Infect 2018. https://doi.org/10.1016/j.cmi.2018.01.007
[36] Rossi B, Barreca F, Benvenuto D, Braccialarghe N, Campogiani L, Lodi A, et al. Human arboviral infections in Italy: past, current, and future challenges. Viruses 2023. https://doi.org/10.3390/v15020368
[37] Kennedy PGE. Viral encephalitis: causes, differential diagnosis, and management. Neurol Pract 2004. https://doi.org/10.1136/jnnp.2003.034280
[38] Calzolari M, Russo S, Marzani K, Dalmonte G, Ricchi M, Bonilauri P. Development of a real-time PCR assay for the detection of the phlebovirus fermo virus. Viruses 2023. https://doi.org/10.3390/v15102082
[39] Daoudi M, Romeo G, Marzani K, Petrella A, Bonilauri P, Lelli D, et al. New isolation of Ponticelli III virus (Bunyavirales: Phenuiviridae) in Emilia-Romagna Region, Italy. Viruses 2023. https://doi.org/10.3390/v15020422