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Original Article

Exploring case definitions and the natural history of respiratory syncytial virus in adult outpatients: First-season results of the RESPIRA-50 study

Alexander Domnich [a,*], Francesco Lapi [b], Andrea Orsi [a,c,d], Piero Luigi Lai [c,d], Luca Pestarino [e,f], Pier Claudio Brasesco [f], Marta Vicentini [g], Anna Puggina [g], Alen Marijam [h], Carlo-Simone Trombetta [c], Giada Garzillo [c], Giulia Guarona [c], Federica Stefanelli [a], Valentina Ricucci [a], Donatella Panatto [c,d], Giancarlo Icardi [a,c,d]

[a] Hygiene Unit, San Martino Policlinico Hospital - IRCCS for Oncology and Neurosciences, Genoa, Italy
[b] Health Search, Italian College of General Practitioners and Primary Care, Florence, Italy
[c] Department of Health Sciences (DISSAL), University of Genoa, Genoa, Italy
[d] Interuniversity Research Center on Influenza and Other Transmissible Infections (CIRI-IT), Genoa, Italy
[e] Private General Practice, Genoa, Italy
[f] Medicoop Liguria, Genoa, Italy
[g] GSK, Verona, Italy
[h] GSK, Wavre, Belgium

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ABSTRACT

Background: Data on the natural history of the community-acquired RSV in adult outpatients are limited. It is also unclear whether the existing influenza surveillance platforms based on influenza-like illness (ILI) case definitions are efficient for RSV. The two-season RESPIRA-50 study was established in 2023 to identify an optimal RSV case definition and to explore the natural history of RSV. Here, the first-season results are reported.

Methods: The study was conducted in Genoa (Italy) during the 2023/2024 RSV season. Twenty-four general practitioners were randomized 1:1 to enroll adults aged ≥ 50 years seeking care for acute respiratory infection (ARI) or ILI, respectively. Both syndromes were defined according to the European criteria. All subjects were tested by real-time polymerase chain reaction (RT-PCR) for RSV and other pathogens. RSV-positive adults were followed for up to 30 days.

Results: Of 517 subjects included, 7.0 % [95 % confidence interval (CI): 4.9–9.5 %)] tested positive for RSV. RSV prevalence in the ARI group (8.0 %; 95 % CI: 5.0–12.1 %) was higher than in the ILI group (6.0 %; 95 % CI: 3.5–9.5 %) with an odds ratio of 1.36 (95 % CI: 0.69–2.70). Conversely, positivity for influenza (10.4 % vs 12.4 %) and SARS-CoV-2 (12.4 % vs 16.9 %) were lower in the ARI group and the corresponding ORs were 0.82 (95 % CI: 0.48–1.42) and 0.70 (95 % CI: 0.43–1.15), respectively. The mean duration of an RSV episode was 18.8 ± 8.0 days and two thirds of individuals were prescribed antibiotics. A total of 33.3 % (95 % CI: 18.6–51.0 %) of RSV-positive individuals developed complications, of which bronchitis (13.9 %) and pneumonia (8.3 %) were the most frequent.

Conclusions: Compared with ARI, ILI-based surveillance may underestimate the burden of RSV in community-dwelling adults aged ≥ 50 years. A high proportion of RSV-positive adult outpatients develops complications, which lead to substantial resource consumption.

Introduction

Respiratory syncytial virus (RSV) is a leading cause of acute respiratory infections (ARIs) in all age groups [1,2]. Although most research on the epidemiology and burden of RSV traditionally focused on young children [3], an increasing number of studies

* Correspondence to: Hygiene Unit, San Martino Policlinico Hospital - IRCCS for Oncology and Neurosciences, Largo Rosanna Benzi 10, Genoa 16132, Italy.
E-mail address: alexander.domnich@hsanmartino.it (A. Domnich).

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pointed out a significant impact of RSV on the morbidity and mortality outcomes in (older) adults [4–6]. For instance, a recent global-level meta-analysis [4] has estimated that as many as 336,000 hospitalizations and 14,000 in-hospital deaths related to RSV occurred in older adults in 2015. A recent availability of the effective vaccines to prevent RSV-related lower respiratory tract disease (LRTD) in older adults [7] can reduce clinical and associated socio-economic burden.

Several features of RSV infection in adults are still to be better understood. First of all, contrary to seasonal influenza, there is no a universally accepted clinical case definition that prompts swab collection for RSV testing and previous studies used a variety of syndromic definitions for the case ascertainment [8]. Some studies have suggested [9–11] that the surveillance systems based on influenza-like illness (ILI) definitions, which have been specifically developed for influenza, underestimate RSV attack rate, since a substantial proportion of RSV-positive adults have no fever. However, the available studies were retrospective, in which different syndromic definitions used to predict RSV positivity were reconstructed from a limited set of available symptoms. An accurate RSV case definition is of primary importance for both routine surveillance activities and future vaccine effectiveness studies. While wider RSV case definitions (e.g., ARI or the presence of any respiratory symptom) are sufficiently sensitive to capture most symptomatic adults, their low specificity may hamper future vaccine effectiveness studies [11].

Other data gaps concern several aspects of the natural history of a community-acquired episode of RSV infection in adults. These, for example, include rate and spectrum of complications, frequency and types of healthcare resource use and associated costs [12]. The recent availability of adult RSV vaccines calls for their evaluation within the framework of the health technology assessment (HTA), which among other domains includes also pharmacoeconomic analyses [13]. Considering that the epidemiology and burden of RSV varies significantly from country to country [4], the cost-effectiveness models should ideally use local epidemiological estimates. In this regard, a recent systematic review [12] has underscored that several features of the progression of a community-acquired RSV infection among Italian adults, such as complication and hospitalization rates, are missing. Even in the international context, this type of data is limited [4–6].

A two-year prospective RESPIRA-50 (RESPIRatory syncytial virus in Adults aged 50 years or more) study has been recently established to address the above-mentioned knowledge gaps on the optimal RSV case definition, to compare the performance of two commonly used case definitions (i.e., ARI and ILI) for RSV surveillance and to investigate the natural history of RSV infection in adult outpatients aged ≥ 50 years. In this paper, the first-season (2023/2024) results are reported.

Materials and methods

Study design

RESPIRA-50 is prospective community-based study conducted in the Metropolitan City of Genoa (Liguria, northwestern Italy). In order to avoid results driven by a single-season epidemiology of RSV, the study was planned to be conducted during two consecutive RSV seasons (2023/2024 and 2024/2025). The RSV season was defined as a period from week 42 (mid-October) to week 17 (end of April).

For the 2023/2024 season, 24 general practitioners (GPs) were randomized on the 1:1 basis in two groups to enroll subjects seeking care for an episode of ARI or ILI, respectively. All GPs completed a training module on all aspects of the study protocol.

The study was approved by the Liguria Region Ethics Committee (Prot. #13354 of September 18, 2023). All subjects provided written informed consent.

Eligibility criteria

Individuals aged ≥ 50 years, regardless of their health conditions, seeking care for an episode of ARI or ILI were potentially eligible. Both syndromes were defined according to the European criteria [14]. In particular, ARI was defined as a sudden onset of ≥ 1 symptom (cough and/or sore throat and/or shortness of breath and/or coryza with or without fever) and clinical assessment of an underlying infection. ILI was defined as follows: sudden onset of ≥ 1 systemic (fever or feverishness, malaise, headache, myalgia) and ≥ 1 respiratory (cough, sore throat, shortness of breath) symptoms.

The following were exclusion criteria: (i) individuals living in long-term care facilities; (ii) previous (current season) positivity for RSV and (iii) swab delay (period between the onset of symptoms and GP visit) > 7 days.

Study procedures

All eligible patients signed the informed consent form, underwent a nasopharyngeal swab collection (which was eluted in the universal transport medium; Copan Italia; Brescia, Italy) and baseline assessment. Relevant demographic (sex, age and nationality), medical history (presence of underlying health conditions) and anamnestic (contact with symptomatic children and adults within the previous eight days) data were gathered. GPs then collected systemic (fever/feverishness and measured body temperature, shivering, headache, myalgia, arthralgia, malaise, decreased appetite, nausea, diarrhea) and respiratory [new onset or increased cough with or without sputum, dyspnea, rhonchi and wheezing; tachypnea (≥ 20 respirations/min); need for oxygen supplementation; low or decreased oxygen saturation (SaO_2 saturation < 95 % or ≤ 90 % if baseline is < 95 %); sore throat; sneezing; nasal congestion/rhinorrhea; altered smell or taste] signs and symptoms relative to the current ARI/ILI episode.

Subjects tested positive for RSV were followed for up to 30 days after the onset of symptoms. The first follow-up call took place 14 ± 1 days after the onset of symptoms with the aim to collect data on further GP and specialist visits, medications, diagnostic tests, complications, emergency department referrals, hospitalizations and working days lost (for working adults). Information provided by the patient was eventually cross-checked with that provided by his or her GP. Complications were determined on the basis of eventual diagnostic tests and/or specialist visits or clinically following development of further symptoms or worsening of the initially reported symptoms with prescriptions of further drugs (antibiotics *in primis*). Patients who made a full recovery from RSV illness (self-judged as return to the premorbid state) by day 14 ended the study. Patients who did make a full recovery were contacted on day 30 ± 1 and the same information was collected.

Laboratory methods

All swabs were tested in real-time polymerase chain reaction (RT-PCR) performed by means of the Allplex Respiratory Panels 1–4 (Seegene Inc.; Seoul, Korea). This kit is able to detect the following respiratory pathogens: RSV A and B; influenza type A, A(H1N1) pdm09, A(H3N2) and B; adenovirus; enterovirus; metapneumovirus; parainfluenza viruses 1–4; bocaviruses 1–4; seasonal coronaviruses 229E, NL63 and OC43; human rhinovirus; *Streptococcus pneumoniae*; *Bordetella pertussis*; *Bordetella parapertussis*; *Chlamydophila pneumoniae*; *Haemophilus influenzae*; *Legionella pneumophila* and *Mycoplasma pneumoniae*. The diagnostic accuracy of this assay in detecting RSV A and RSV B viruses is 100 % [15]. For SARS-CoV-2, the Allplex SARS-CoV-2/FluA/FluB/RSV assay (Seegene Inc.; Seoul, Korea) was used. Both RT-PCR assays were performed

according to the manufacturer's instructions. Samples with cycle threshold (Ct) values < 40 were deemed positive.

Twenty-nine (81 %) RSV-positive samples were sequenceable. In particular, the next generation sequencing (NGS) was performed to obtain whole genome sequences and was used for samples with Ct < 30, while the remaining specimens were sequenced using the Sanger method to acquire the full G gene sequence. Sequencing was performed according to the protocols described previously [3,16]. All nucleotide sequences generated were submitted to the GISAID (Global Initiative on Sharing All Influenza Data) database (https://gisaid.org/).

Study outcomes

The study has two primary outcomes, namely (i) prevalence of RSV detections in the ARI and ILI groups and (ii) prevalence of complications, including hospital encounters, among all RSV-positive adults. Our secondary and descriptive outcomes included: prevalence of RSV subtypes; prevalence of RSV mono- (detection of only RSV) and co-detections (detection of RSV and other pathogens in the same sample); spectrum of symptoms in the ARI and ILI groups and among individuals tested positive for RSV; RSV-related resource consumption in terms of GP and specialist visits, medicines and diagnostic procedures. The study has also several other secondary and descriptive endpoints (e.g., analysis of subgroups of patients based on latent classes, direct and indirect costs), which will be assessed by the end of the second season.

Statistical analysis

Prevalence of RSV and other categorical variables were expressed as percentages with Clopper-Pearson's exact 95 % confidence intervals (CIs). Proportions were compared by means of the Fisher's exact test and the effect size was expressed as odds ratios (ORs). Continuous variables were expressed as means with standard deviations (SDs) and compared by computing the independent *t* test.

Statistical analysis was performed in R stat packages v. 4.1.0 (R Foundation for Statistical Computing; Vienna, Austria).

Results

Characteristics of the study population

A total of 524 subjects were enrolled. Six subjects were excluded for protocol deviations: four were < 50 years and two were swabbed outside the allowed time window. Additionally, one specimen was discarded from testing, as it was lacking a cotton flock inside the transport tube. Therefore, the analysis was based on 517 individuals. Of these latter, 48.4 % (n = 250) and 51.6 % (n = 267) of individuals were enrolled by GPs in the ARI and ILI groups, respectively.

Two thirds (62.3 %) of the individuals enrolled were women and their mean age was 66.7 (SD 10.6) years. Cardiovascular (34.2 %) and respiratory (19.7 %) diseases were the most frequently recorded. ARI and ILI groups were comparable in terms of sociodemographic characteristics, month of GP visit and prevalence of most underlying health conditions, except for diabetes (11.2 % *vs* 5.2 %; p = 0.015) and rheumatic diseases (2.0 % *vs* 11.2 %; p < 0.001). Moreover, the period between symptom onset and GP visit was on average shorter in the ILI group (3.0 *vs* 3.5 days; p = 0.002) (Table 1). Notably, all patients (100 %; 267/267) in the ILI group met also the definition of ARI, while 96.0 % (240/250) of individuals in the ARI group met also the definition of ILI.

A total of 25.9 % (95 % CI: 22.2–29.9 %) and 37.5 % (95 % CI: 33.3–41.9 %) of patients reported a previous contact with symptomatic children and adults, respectively. While the prevalence of previous contacts with sick children was similar (p = 0.69) between the ARI (26.8 %) and ILI (25.1 %) groups, adult contacts were more frequent in the ILI group (41.6 % *vs* 33.2 %; p = 0.056).

Compared with the ARI group, adults in the ILI group reported more frequently any fever or feverishness (79.0 % *vs* 62.0 %; p < 0.001), nausea (26.6 % *vs* 18.8 %; p = 0.037), sore throat (65.9 % *vs* 56.4 %; p = 0.030), rhonchi (24.0 % *vs* 16.8 %; p = 0.050), sneezing

Table 1
Characteristics of the study population, by surveillance group.

Characteristic	Level	Total (n = 517)	ARI (n = 250)	ILI (n = 267)	p-value
Sex	Female	62.3 (322)	61.6 (154)	62.9 (168)	0.79
	Male	37.7 (195)	38.4 (96)	37.1 (99)	
Age, years	Mean (SD)	66.7 (10.6)	67.3 (10.6)	66.2 (10.6)	0.27
	50–59	28.4 (147)	26.0 (65)	30.7 (82)	0.51
	60–69	35.4 (183)	36.8 (92)	34.1 (91)	
	≥ 70	36.2 (187)	37.2 (93)	35.2 (94)	
Nationality	Italian	93.0 (481)	93.6 (234)	92.5 (247)	0.73
	Foreigner	7.0 (36)	6.4 (16)	7.5 (20)	
Month of swab	Oct 2023	8.1 (42)	8.0 (20)	8.2 (22)	0.55
	Nov 2023	26.5 (137)	26.4 (66)	26.6 (71)	
	Dec 2023	13.7 (71)	14.0 (35)	13.5 (36)	
	Jan 2024	18.2 (94)	17.2 (43)	19.1 (51)	
	Feb 2024	14.3 (74)	16.8 (42)	12.0 (32)	
	Mar 2024	10.3 (53)	10.8 (27)	9.7 (26)	
	Apr 2024	8.9 (46)	6.8 (17)	10.9 (29)	
Swab delay	Mean (SD)	3.2 (1.7)	3.5 (1.7)	3.0 (1.6)	0.002
Underlying health conditions	Cardiovascular	34.2 (177)	33.2 (83)	35.2 (94)	0.64
	Respiratory	19.7 (102)	19.6 (49)	19.9 (53)	0.99
	Diabetes	8.1 (42)	11.2 (28)	5.2 (14)	0.015
	Hepatic	1.4 (7)	2.0 (5)	0.7 (2)	0.27
	Renal	3.9 (20)	5.2 (13)	2.6 (7)	0.17
	Rheumatic	6.8 (35)	2.0 (5)	11.2 (30)	< 0.001
	Anemia	4.1 (21)	5.2 (13)	3.0 (8)	0.27
	Dementia	2.1 (11)	2.4 (6)	1.9 (5)	0.77
	Cancer	9.3 (48)	7.2 (18)	11.2 (30)	0.13
	Immunosuppression	3.7 (19)	3.6 (9)	3.7 (10)	0.99
Smoking habits	Never smoked	56.9 (294)	56.8 (142)	56.9 (152)	0.51
	Former smoker	27.5 (142)	29.2 (73)	25.8 (69)	
	Current smoker	15.7 (81)	14.0 (35)	17.2 (46)	

ARI; Acute respiratory infection; ILI: Influenza-like illness; SD: Standard deviation.

(73.4 % *vs* 64.8 %; p = 0.036) and altered smell (19.1 % *vs* 12.4 %; p = 0.041) (Table S1).

Prevalence of RSV and other respiratory pathogens in the ARI and ILI surveillance groups

Overall, RSV was detected in 7.0 % (95 % CI: 4.9–9.5 %; 36/517) of adults. RSV prevalence in the ARI group (8.0 %; 95 % CI: 5.0–12.1 %; 20/250) was two percent points higher than in the ILI group (6.0 %; 95 % CI: 3.5–9.5 %; 16/267) with an OR of 1.36 (95 % CI: 0.69–2.70). The difference between the ARI and ILI groups was not significant (p = 0.39). Notably, all 36 RSV-positive individuals met both ARI and ILI case definitions.

More generally, ≥ 1 pathogen was identified in 76.2 % (394/517) of individuals and these proportions were similar between the ARI (75.2 %) and ILI (77.2 %) groups (Table S2). The remaining 23.8 % (123/517) tested negative for all viral and bacterial pathogens. Apart from RSV, other common respiratory viruses were rhinovirus (19.7 %), SARS-CoV-2 (14.7 %), influenza (11.4 %) and metapneumovirus (8.3 %). Among bacteria, *Haemophilus influenzae* (19.0 %) and *Streptococcus pneumoniae* (4.4 %) were the most common. Contrary to RSV, positivity for influenza (10.4 % *vs* 12.4 %) and SARS-CoV-2 (12.4 % *vs* 16.9 %) were lower in the ARI than in the ILI groups and the corresponding ORs were 0.82 (95 % CI: 0.48–1.42) and 0.70 (95 % CI: 0.43–1.15). The between-group differences were not significant for both influenza (p = 0.49) and SARS-CoV-2 (p = 0.17).

Among RSV-positive samples, 52.8 % (19/36) samples were RSV mono-detections. The remaining 47.2 % of samples were co-detected with ≥ 1 other pathogen, of which colonization with *Haemophilus influenzae* was the most frequent (Table S3). Prevalence of RSV mono-detections was 4.8 % (95 % CI: 2.5–8.2 %; 12/250) in the ARI group and 2.6 % (95 % CI: 1.1–5.3 %; 7/267) in the ILI group with an OR of 1.87 (95 % CI: 0.73–4.84).

Epidemiology of RSV in the 2023/2024 season

Most (86.1 %; 31/36) RSV cases were detected between January and February 2024 (Fig. 1A). Indeed, in January [ARI: 20.9 % (9/43); ILI: 15.7 % (8/51)] and February [ARI: 19.0 % (8/42); ILI: 18.8 % (6/32)] about one fifths of all samples tested positive for RSV. Compared with the ILI group, the observed detection curve in the ARI group was shifted to the left (Fig. 1B).

Both RSV subtypes A (61.1 %; 22/36) and B (38.9 %; 14/36) co-circulated during the season. Within molecularly characterized RSV A samples (n = 17), 47.1 %, 23.5 %, 11.8 %, 11.8 % and 5.9 % adjoined subclades A.D.1, A.D.5.1, A.D.5.2, A.D.3 and A.D.5, respectively. The population of RSV B (n = 12) was represented by subclades B.D.E.1 (66.7 %) and B.D.4.1.1 (33.3 %) (Table S4).

Clinical features and natural history of RSV

Among RSV-positive individuals (n = 36), malaise (88.9 %) was the most common systemic symptom. Fever or feverishness were present only in 63.9 % of subjects. The most common respiratory symptoms included any cough (94.4 %), sneezing (80.6 %) and cough with sputum (75.0 %). Wheezing was present in one quarter (25.0 %) of subjects (Table 2). Compared with RSV-negative subjects (n = 481), those who tested positive for RSV had a higher prevalence (75.0 % *vs* 55.9 %; p = 0.035) of cough with sputum (Table S5).

The average duration of an RSV episode was 18.8 (SD 8.0) days and during this period patients visited their GPs 1.5 (SD 0.7) times. All but one (97.2 %) individual took ≥ 1 medicine due to the current RSV episode and two thirds (61.1 %) were prescribed ≥ 1 antibiotic. Cumulatively, 7 (19.4 %) patients performed 12 diagnostic procedures, of which chest X-ray was the most frequent (50.0 %). Additionally, four (11.1 %) individuals performed a specialist visit.

As shown in Table 3, one third of individuals (33.3 %; 95 % CI: 18.6–51.0 %) developed ≥ 1 complication. Pneumonia was recorded in 8.3 % (3/36) of patients. There was one hospitalized case (2.8 % to RSV-positive subjects and 8.3 % to complicated cases) that concerned a 71-year-old woman who developed pleural effusion. Her sample tested positive for RSV B and *Haemophilus influenzae*. No deaths were recorded during the follow-up.

Discussion

To our knowledge, RESPIRA-50 is the first randomized study aimed at comparing frequency of RSV detection among outpatient

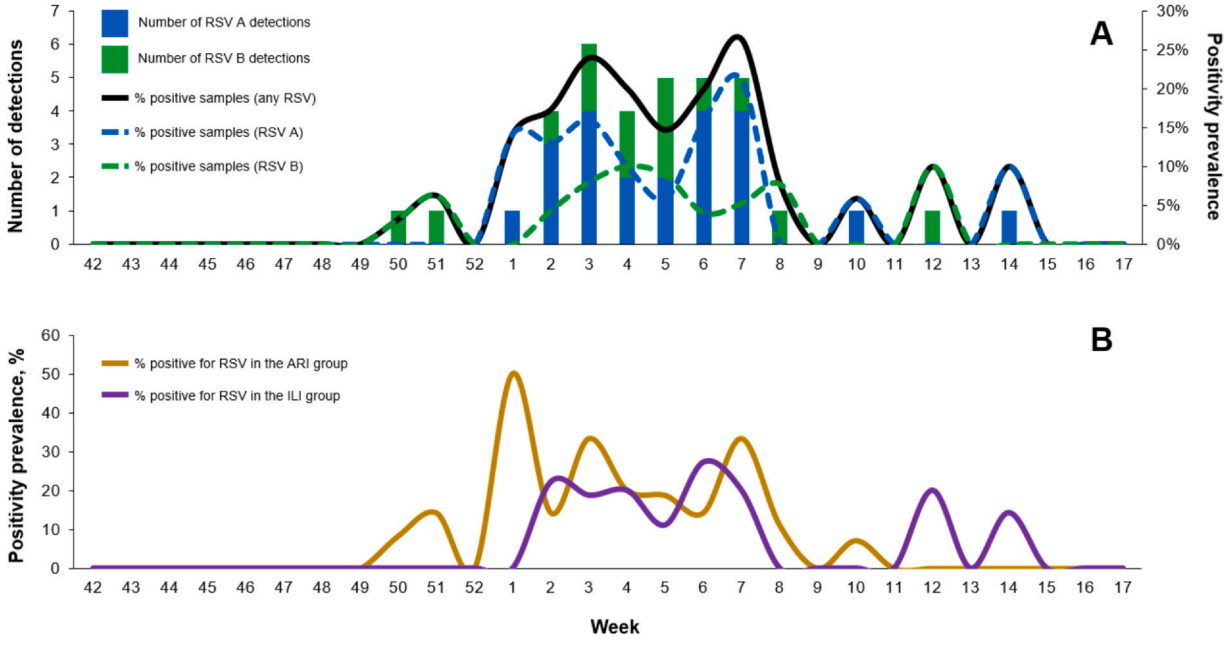

Fig. 1. Weekly distribution of samples positive for respiratory syncytial virus (RSV) by subtype (A) and surveillance group (B). ARI, acute respiratory infection; ILI, influenza-like illness.

Table 2
Frequency of symptoms among Italian adults aged ≥ 50 years who tested positive for respiratory syncytial virus (RSV).

Symptom		% (n)	
		Any RSV (n = 36)	RSV mono-detections (n = 19)
Systemic	Fever/feverishness	63.9 (23)	57.9 (11)
	37–37.9 °C	40.9 (9)[a]	45.5 (5)
	38–38.9 °C	45.5 (10)[a]	54.5 (6)
	≥ 39 °C	13.6 (3)[a]	0 (0)
	Shivering	63.9 (23)	47.4 (9)
	Headache	50.0 (18)	52.6 (10)
	Myalgia	55.6 (20)	52.6 (10)
	Arthralgia	50.0 (18)	42.1 (8)
	Malaise	88.9 (32)	89.5 (17)
	Decreased appetite	55.6 (20)	47.4 (9)
	Nausea	22.2 (8)	21.1 (4)
	Diarrhea	16.7 (6)	21.1 (4)
Respiratory	Any cough	94.4 (34)	100 (19)
	Cough with sputum	75.0 (27)	89.5 (17)
	Dyspnea	25.0 (9)	56.3 (5)
	Rhonchi	25.0 (9)	21.1 (4)
	Wheezing	25.0 (9)	21.1 (4)
	Tachypnea	0 (0)	0 (0)
	Need for O2	0 (0)	0 (0)
	Low/ decreased SaO2	5.6 (2)	5.3 (1)
	Sore throat	55.6 (20)	47.4 (9)
	Sneezing	80.6 (29)	73.7 (14)
	Nasal congestion/ rhinorrhea	66.7 (24)	68.4 (13)
	Altered smell	25.0 (9)	15.8 (3)
	Altered taste	22.2 (8)	15.8 (3)

[a] Measured body temperature in patients with fever or feverishness was unavailable for one subject.

Table 3
Frequency of complications among Italian adults aged ≥ 50 years who tested positive for respiratory syncytial virus (RSV).

Complication	% (n)	
	Any RSV (n = 36)	RSV mono-detections (n = 19)
Any	33.3 (12)	31.6 (6)
Any upper respiratory tract complication	13.9 (5)	15.8 (3)
Sinusitis	8.3 (3)	15.8 (3)
Otitis	5.6 (2)	0 (0)
Any lower respiratory tract complication	25.0 (9)	26.3 (5)
Bronchitis	13.9 (5)	15.8 (3)
Pneumonia	8.3 (3)	10.5 (2)
Pleural effusion	2.8 (1)	0 (0)

adults seeking care for ARI or ILI. In the Italian context, it is among the first studies [12] that went beyond a pure quantification of RSV positivity prevalence in adult outpatients and was able to investigate the natural history of RSV infection, such as case-complication rate. The first-season results suggest a clear trend for a higher RSV detection rate in ARI patients and therefore ILI-based surveillance systems may underestimate the burden of RSV in community-dwelling adults aged ≥ 50 years. We finally found one third of RSV-positive adults aged ≥ 50 years developed respiratory complications, which led to a substantial resource use.

Although the null hypothesis could not be rejected (likely because of a limited sample size), the odds of testing positive for RSV in the ARI versus ILI groups increased by 36 %. This prospectively derived estimate is in line with some previous retrospective analyses [9–11,17], which concluded that ILI- and fever-based syndromic definitions are less sensitive for RSV and may underestimate its attack rate. In our study, only two thirds (63.9 %) of individuals had a history of fever or feverishness, while the most common symptoms were any cough (94.4 %), malaise (88.9 %), sneezing (80.6 %) and cough with sputum (75.0 %). Analogous figures were documented in a Portuguese study [10] that reported a prevalence of fever/feverishness of 65.4 % and 53.3 % in adults aged 15–64 and ≥ 65 years, respectively. Similarly to our findings, cough was the most common symptom in both younger (92.5 %) and older adults (97.8 %) [10]. As we mentioned earlier, previous retrospective analyses leveraged data from the existing influenza surveillance programs and therefore the list of available symptoms was limited to ILI case definitions. The RESPIRA-50 study envisaged a more comprehensive list of systemic and respiratory signs and symptoms and, providing a sufficient number of RSV cases in the second season, we hope to apply latent class models to identify symptomatic patterns in the clinical presentation of RSV infection in adults.

Compared with the ILI group, the first RSV detections in the ARI group occurred some weeks earlier. If it did not occur by chance, the observed shift could be driven by the fact that the ARI surveillance is more sensitive to capture RSV cases with mild clinical picture, as the definition of ARI does not require presence of systemic symptoms. In any case, this finding should be further confirmed.

Most available studies on the evolution of a community-acquired RSV episode in (older) adults were conducted in hospital settings [12,18] and only few data on case-complication rates are available. A comprehensive review by Falsey and Walsh [19] reported that up to 10 % of RSV-positive adults develops pneumonia, which is consistent with our finding (8.3 %). Analogously, in a 12-season United States (US) study [20], 9.5 % of older adults aged ≥ 60 years developed pneumonia. The occurrence of other than pneumonia complications was less studied. Bruyndonckx *et al.* [21] documented that during a 28-day follow-up, 22 % of RSV-positive adults experienced some disease deterioration, which was defined as re-consultation with new or worsened complaints or hospitalization. Assuming comparability, in our study the case-complication rate was slightly higher (33 %), probably because the study population was significantly older.

In our study, one hospitalization attributable to RSV was recorded during the follow-up. A single occurrence prevented us from calculating hospitalization rate. Contrary to the frequency of pneumonia, the currently available estimates for RSV-related hospitalization rates seem to be less homogeneous. For instance, in the abovementioned US study [21], the hospitalization rate was 10.6 %. In another US study [22] on adults aged ≥ 50 years, the rate of hospital encounters was lower (7.0 %). Falsey *et al.* [23] recorded hospitalization rates of 0 % in healthy adults aged ≥ 65 years and 16.1 % in high-risk adults aged ≥ 21 years.

About one half of RSV-positive individuals were co-detected (27.8 % and 30.6 % had at ≥ 1 viral and bacterial co-detection, respectively) with other respiratory pathogens, of which *Haemophilus influenzae* was the most prevalent. Although our study design of only symptomatic individuals who provided a single swab does not allow to differentiate between temporary colonization or true bacterial infection, it is evident that a substantial proportion of *Haemophilus influenzae* detections (especially if co-detected with respiratory viruses) is an occasional colonization. Nasopharyngeal carriage of *Haemophilus influenzae* is common: an Italian study among adults aged ≥ 50 years [24] showed an overall (in both symptomatic and asymptomatic adults) prevalence of 10.5 %. When considered only individuals with respiratory symptoms, prevalence of *Haemophilus influenzae* detection raised to 17.7 % [24], which is consistent with our finding. Some previous research has shown that co-infections are common among RSV-positive adults [3,25] and may lead to worse clinical outcomes [26]. It should be, however, stressed that the prevalence of RSV co-detections is highly variable and depends on the number of pathogens tested. For instance, if the so-called "essential" multiplex respiratory panels (e.g., RSV, influenza and SARS-CoV-2) are used, the prevalence of co-detections is intrinsically

underestimated. Interestingly, we also noted a signal that the co-detection rate was higher in the ILI group. Compared with RSV mono-infections, subjects with co-detections had higher prevalence of fever or feverishness (57.9 % vs 70.6 %, results not shown). Although these data are based on small numbers and should be further confirmed, we suppose that ARI-based surveillance may be more sensitive to capturing cases due to milder RSV illness without systemic signs and symptoms.

We must acknowledge some study important limitations. First, a relatively small number of RSV-positive samples determined wide 95 % CIs. On the other hand, the observed effect size supports the idea of a higher prevalence of RSV in ARI patients. A limited sample size during the first season did not also allow us to perform other analyses, including calculation of sensitivity, specificity and predictive values of alternative RSV case definitions. As the RESPIRA-50 study will continue enrolling patients during the next 2024/2025 season, we hope to obtain more precise estimates. Second, although the ARI and ILI groups were mostly comparable, some differences in the prevalence of diabetes and rheumatic diseases were observed. Some covariance imbalance is likely to occur in cluster randomization studies, as the number of clusters is comparatively low (24 in our case) [27]. Analogously, some GPs had a second specialty and therefore their patient lists could include a higher number of subjects affected by these co-morbidities. Finally, being case-ascertainment in nature, the study enrolled only symptomatic individuals. This study was also not designed as a cohort study to estimate incidence of symptomatic RSV, which would require frequent follow-up (including zero reporting) to avoid missing symptomatic infections. Large community-based prospective cohort studies are warranted.

In conclusion, this study showed that during the 2023/2024 winter season in Italy, prevalence of RSV detection was 7.0 %, which higher than average [12]. Compared with ILI patients, RSV detection rates tended to be higher among those with ARI and therefore historical influenza surveillance platforms leveraged for RSV may underestimate burden of the former. Indeed, available retrospective studies [9–11] documented that ILI-based surveillance systems may underestimate the burden of RSV in older adults. This prospective study adds to that body of evidence. Following a positive RSV test, as many as one third of outpatient adults aged ≥ 50 years develop complications, which further weighs on healthcare systems.

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Declaration of Competing Interest

A.D. provided consultancies and received speaker fees from CSL Seqirus, GSK and SD Biosensor. A.O. provided consultancies and/or received speaker fees from CSL Seqirus, Moderna, Novavax and SD Biosensor. M.V., A.P. and A.M. are employees of the GSK group of companies and may hold shares in the GSK group of companies as part of their employee remuneration. D.P. provided consultancies for Pfizer and CSL Seqirus and received grants for conducting observational studies from Sanofi, Pfizer, GSK and Viatris. G.I. provided consultancies and/or received grants for conducting experimental and/or observational studies for GSK, Sanofi, MSD, CSL Seqirus and Pfizer. Other authors declare no conflicts of interest.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jiph.2025.102653.

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