Journal of Public Health: From Theory to Practice (2022) 30:599–606
https://doi.org/10.1007/s10389-020-01333-9

ORIGINAL ARTICLE

Influenza molecular diagnostic testing in a 1000-bed academic Italian hospital during the 2018–19 influenza season

Francesca Valent[1] · Francesca Malacarne[1,2] · Sabrina Licata[1] · Corrado Pipan[1,2]

Received: 6 April 2020 / Accepted: 5 June 2020 / Published online: 16 June 2020

Abstract

Aim The aims of this study were to examine the requests for influenza molecular tests processed by the Virology Laboratory of the University Hospital of Udine during the 2018–19 influenza season and to assess the test results and to estimate costs.

Subjects and methods We analyzed various administrative databases of the hospital health information system, which can be deterministically linked at the individual level through an anonymous stochastic key. Requests for influenza molecular tests from November 1, 2018, to April 15, 2019, and test results were described by week and, for hospitalized patients, hospital ward. Previous vaccination status of tested patients, outcomes and estimated test costs were assessed.

Results In the 2018–19 influenza season, 979 influenza A and B virologic tests were processed by the laboratory, corresponding to 758 patients. Requests had more than doubled compared with the previous influenza season. Rapid real-time PCR tests, routinely available at the University Hospital of Udine since January 2019, represented 17% of requests. Six hundred forty-eight patients were hospitalized. Medical wards requested the test after a median of 1 day after admission, whereas requests were delayed for surgical and oncologic patients. The number of tests, proportion of positivity and consumption of rapid tests varied by medical specialty. Overall consumption of oseltamivir was similar to that of the previous influenza season.

Conclusions This analysis, benefiting from the availability of integrated health administrative databases, provided useful information to support public health decision-making and managing the supply and demand for diagnostic tests.

Keywords Influenza · Real-time PCR · Virology · Administrative data · Italy

Introduction

Laboratory diagnosis of influenza is important for several reasons. The laboratory can isolate circulating viruses and contribute to vaccine development; in addition, thanks to the ability to differentiate virus subtypes with similar clinical presentation, it has a role in disease surveillance and treatment of illness. In the hospital setting, where the treatment aim is predominant, prompt and accurate diagnosis supports clinical decisions regarding infection control measures and antimicrobial therapy.

In the Italian Northeastern Region Friuli Venezia Giulia, clinical recommendations for the management of hospitalized patients with influenza-like syndromes (Regione autonoma Friuli Venezia Giulia 2019) require that biological samples are collected from patients with serious or complicated illness within 3–4 days from the start of symptoms and that they are sent to a reference laboratory for influenza confirmation, subtyping and mutation detection. However, the decision regarding whether to initiate antiviral therapy is based only on clinical severity of illness and individual risk factors, according to the guide issued by the Centers for Disease Control and Prevention (CDC 2019). Nonetheless, as the CDC remarks, hospitalized patients with suspected influenza should be tested with molecular assays with high sensitivity and specificity (such as real-time PCR) to inform decisions on antiviral therapy and to prevent nosocomial outbreaks through prompt implementation of control measures (CDC 2019).

The Italian integrated epidemiologic and virologic influenza surveillance system InfluNet showed that, after one of the highest peaks of the past 15 years registered in the 2017–18

✉ Francesca Valent
francesca.valent@asufc.sanita.fvg.it

[1] Unit of Hygiene and Clinical Epidemiology, University Hospital of Udine, Via Colugna 50, 33100 Udine, Italy

[2] Department of Medical Area, University of Udine, Udine, Italy

season, the incidence of influenza-like syndromes in the 2018–19 season was slightly lower than in the previous year (InfluNet 2019).

In the University Hospital of Udine, a tertiary 1000-bed academic hospital located within the Friuli Venezia Giulia region, influenza virus testing through molecular assays can be requested of the Virology Laboratory, where, since January 2019, two rapid molecular assays, with test time < 30 min, have been available in addition to multiplex molecular assays, with test time of hours. According to the hospital's internal procedures, rapid assays should be requested for hospitalized patients only upon an infectious disease consult.

The principal objectives of this study were to examine the requests of influenza molecular tests processed by the Virology Laboratory of the University Hospital of Udine during the 2018–19 influenza season to assess test results and to estimate costs.

Methods

We used the administrative databases of the Health Information System of the Local Health Authority of Udine as the source of information. Databases are anonymous (patients cannot be identified); however, they can be linked with each other at the individual patient level through a univocal stochastic key.

For this study, we analyzed the laboratory database, which includes requests and results of all laboratory tests conducted at the University Hospital of Udine, located in the territory served by the Local Health Authority of Udine, the hospital discharge database, the hospital drug consumption database and the vaccination database of the Local Health Authority of Udine.

We analyzed all the influenza A and B molecular diagnostic tests performed by the Virology Laboratory of the Hospital between November 1, 2018, and April 15, 2019. The available tests are:

- Allplex™ Respiratory Panel 1 (Seegene, Republic of Korea), a one-step real-time PCR assay for influenza A virus, influenza B virus, respiratory syncytial virus A, respiratory syncytial virus B, influenza A-H1, influenza A-H1pdm0, influenza A-H3 on nasopharyngeal swab, nasopharyngeal aspirate or bronchoalveolar lavage, available throughout the entire 2018–19 season as well as in the previous year;
- Xpert®Flu (Cepheid, USA), a rapid real-time PCR assay for influenza A, B and H1N1 on nasal aspirate/washes or nasopharyngeal, routinely available in Udine since January 2019;
- Xpert® Xpress Flu/RSV (Cepheid, USA), a rapid real-time PCR assay for influenza A and B and respiratory syncytial virus on nasal aspirate/washes or nasopharyngeal, routinely available in Udine since January 2019.

Two additional multiplex tests for respiratory infections, not including influenza virus, but often requested at the same time, are:

- Allplex™ Respiratory Panel 2 (Seegene, Republic of Korea), for adenovirus, enterovirus, metapneumovirus, parainfluenza virus 1, parainfluenza virus 2 and parainfluenza virus 3;
- Allplex™ Respiratory Panel 3 (Seegene, Republic of Korea), for bocavirus 1/2/3/4, coronavirus 229E, coronavirus NL63, coronavirus OC43 and rhinovirus.

We assessed the frequency of tests requested in each week of the study period, of patients with at least one influenza test and of patients with at least one positive test in the season.

For tests requested for patients admitted to the hospital, with sampling date between admission and discharge dates, we described the specialty of the ward where the patient was hospitalized, timing of the request, main discharge diagnoses and hospitalization outcome.

In all patients with influenza tests, we assessed whether they had been vaccinated at least 14 days before the influenza test sampling. Differences in the frequency of vaccinated patients in the group with negative test results and those with positive results were assessed through the chi-square test. The statistical significance of differences in continuous numerical variables was assessed using the t-test in case of variables with a normal distribution and Wilcoxon's rank sums test otherwise. Normality was checked using the Kolmogorov-Smirnov test. P values < 0.05 were considered statistically significant.

We compared the number of test requests and estimated costs in the 2018–19 season with those in the 2017–18 season, when the rapid test was not routinely available in Udine. The number of doses of the antiviral drug oseltamivir (ATC code J05AH02) distributed to the wards by the hospital pharmacy during the two influenza seasons was also described.

All the analyses were conducted using SAS v7.15 (SAS Institute Inc., Cary, NC, USA).

Ethical considerations

All procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Declaration of Helsinki. The analyses were based on anonymous administrative data; therefore, patient informed consent and ethics committee approval were not required in Italy. Patients were not identifiable, and their privacy rights were always observed.

Results

During the 2018–19 influenza season, 979 influenza A and B virologic tests were processed by the Virology Laboratory of the University Hospital of Udine, corresponding to 758 patients. Of them, 618 (81.5%) had only one test request during the season whereas the others had two or more (up to 5). Overall, 814 traditional tests and 165 rapid tests were conducted.

Eight hundred nine requests (82.6%) were related to 648 inpatients hospitalized at the University Hospital of Udine. The trends of influenza virus test requests among inpatients are shown in Fig. 1. Traditional tests outnumbered rapid tests; from week 3 to week 8 of 2019, rapid molecular assays represented > 20% of all requests. Overall, the peak of requests was from week 4 to week 9 of 2019.

Thirty-two hospital wards requested at least one influenza virus test during the 2018–19 season. Specialties with the highest number of requests were internal medicine (3 wards, overall 28.7% of all tests), infectious diseases (13.7% of all tests), pneumology (11.7%), hematology (9.5%), pediatrics (8.7%), intensive care units (3 wards, 5.7%) and oncology (4.2%), accounting for > 80% of all requests. Rapid tests were requested by 19 wards. Figure 2 shows the proportion of rapid tests for specialties requesting ≥ 10 tests. The average number of test requests per hospitalized patient ranged from values close to 1 for emergency medicine, nephrology, pediatrics and neonatology to values > 1.5 for cardiac surgery, ICU and general surgery.

Overall, median time from admission to testing was 1 day (25th percentile 1 day, 75th percentile 6). Median time from admission to testing ranged from 1 day in most medical specialties (internal medicine, cardiology, rheumatology, emergency medicine, infectious diseases, neonatology, pediatrics, pneumology) to 5–8 days in most surgical specialties (except obstetrics and gynecology, 1 day) to more than 1 week for onco-hematology. In seven hospitalizations with length of stay > 1 day, the test was requested for the first time on the same day as the patient's discharge.

None of the 648 tested inpatients were positive for influenza B virus; 114 (17.2%) were positive for influenza A virus. Positivity for influenza A virus among inpatients is shown in Fig. 3. Positivity started in the first week of 2019 and ended at week 12. The proportion of influenza-A positivity by specialty is shown in Fig. 4.

Of the 758 patients who underwent influenza A virologic tests in the 2018–19 season, 188 (24.8%) had been vaccinated against influenza at least 14 days before being tested. Tests

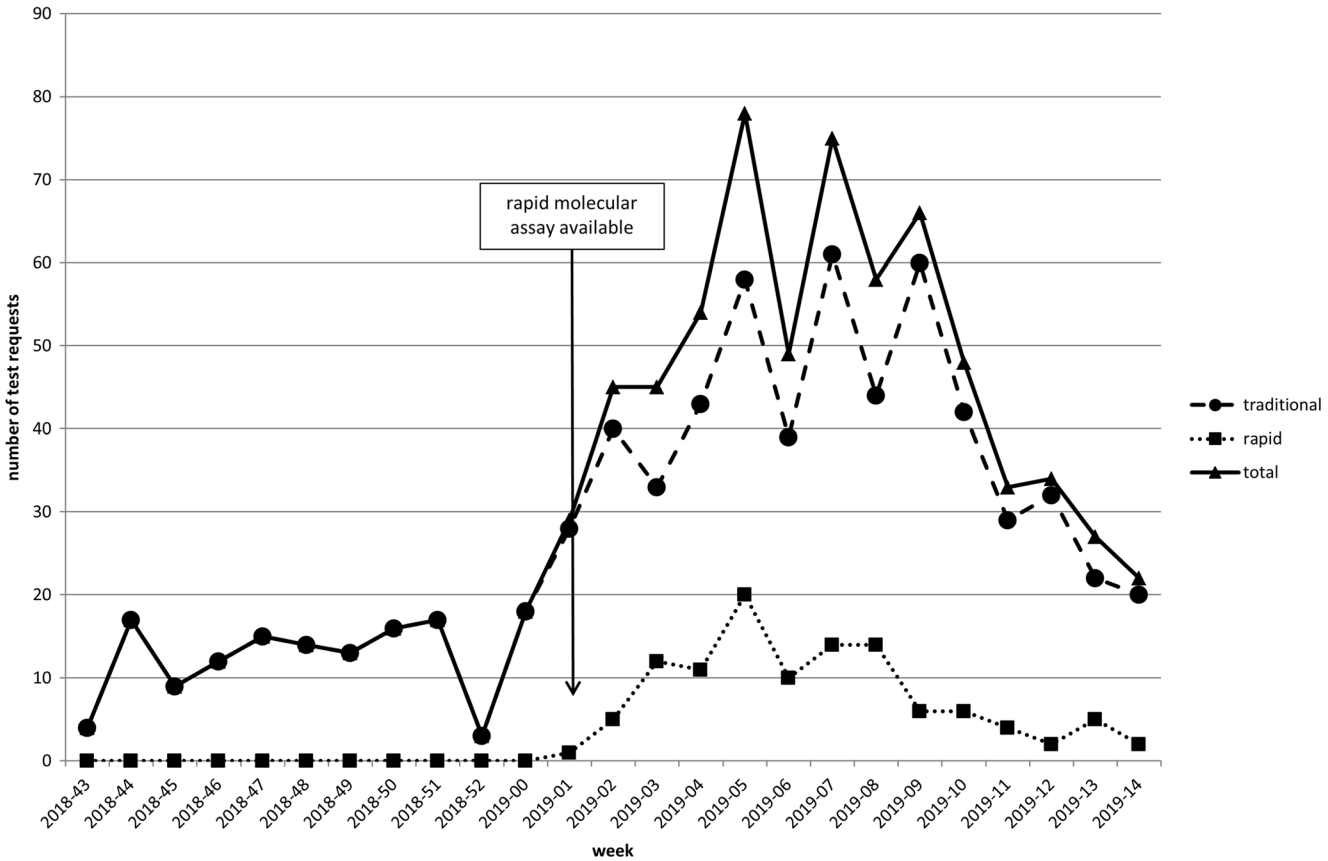

Fig. 1 Requests for influenza virus tests from November 1, 2018, to April 15, 2019, among patients hospitalized at the University Hospital of Udine, Italy

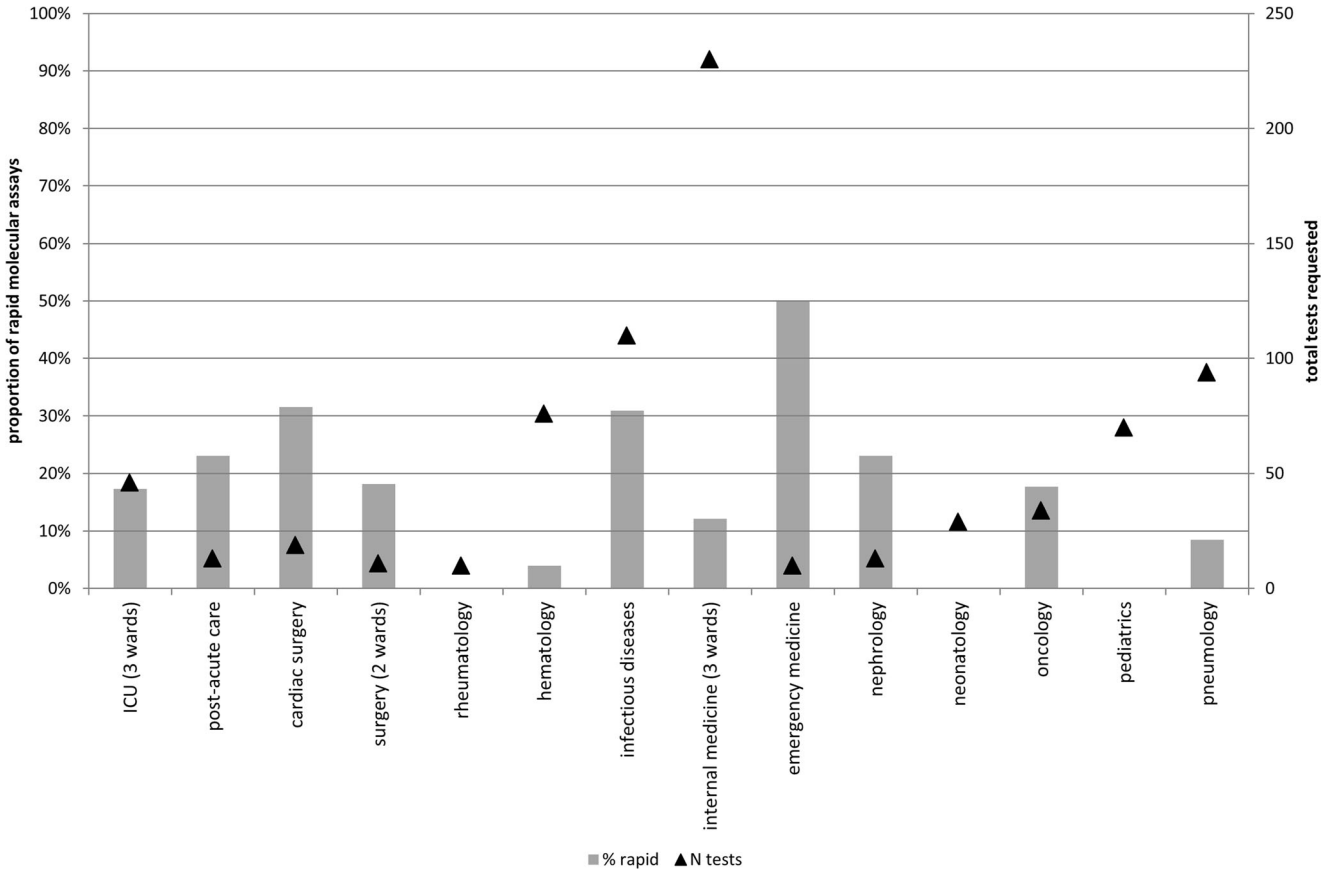

Fig. 2 Number of influenza virus tests and proportion of rapid molecular assays among patients admitted to the University Hospital of Udine, Italy, during the 2018–19 influenza season, by medical specialty

were positive in 18.8% of unvaccinated and 21.3% of vaccinated patients ($p = 0.4513$). After stratifying by age (< 65 vs. ≥ 65 years), positivity was almost identical in unvaccinated and vaccinated subjects (16.4% vs. 15.6%, $p = 0.8818$, in the younger group and 22.4% vs. 23.1%, $p = 0.8838$, in the elderly). Among influenza A-positive inpatients, 28 (24.6%) were assigned a main discharge diagnosis of influenza (ICD-9-CM 487.x). Thirteen influenza A-positive inpatients (11.4%) died during the hospitalization; in-hospital crude mortality among influenza A-negative inpatients was 10.1%. Length of stay was similar in influenza-A-positive and -negative patients (mean 15.8 ± 12.3, median 12 days, mean 18.9 ± 21.5, median 11, respectively; p value of Wilcoxon's rank sums test 0.7260).

In the 2017–18 season, overall requests for influenza A and B virus tests at the University Hospital of Udine were 426 in 375 patients; 4.5% of tests were positive for influenza A virus and 9.9% for influenza B. In the 2017–18 season, all wards requested fewer tests than in 2018–19, except pediatrics (77 requests in 2017–18 vs. 70 in 2018–19).

The estimated cost of diagnostic tests, assuming conservatively to have used 9 multiplex panel 100-test kits (with a 70–75% yield) and 17 rapid 10-test kits in the 2018–19 season and 5 multiplex panel 100-test kits in the 2017–18 season, was 14,127 euros in 2018–19 and 4165 euros in 2017–18. The cost per detected case was 77.6 euros and 68.3 euros, respectively.

In the 2018–19 season, 1815 doses of oseltamivir were distributed to the wards by the hospital pharmacy (10 in November, 72 in December, 861 in January, 434 in February, 355 in March and 83 in the first half of April). In the corresponding period of the 2017–18 season, doses were 1616. Consumption of oseltamivir by medical specialty is shown in Table 1.

Discussion

Main finding of this study

This study described the requests for molecular diagnostic tests for influenza virus processed by the Virology Laboratory of the Italian 1000-bed University Hospital of Udine during the 2018–19 influenza season, when two rapid real-time PCR assays were made available in addition to standard multiplex real-time PCR assays.

In the 2018–19 season, although in Italy the incidence of influenza-like syndromes was similar to that of the previous season (InfluNet 2019), the number of influenza test requests

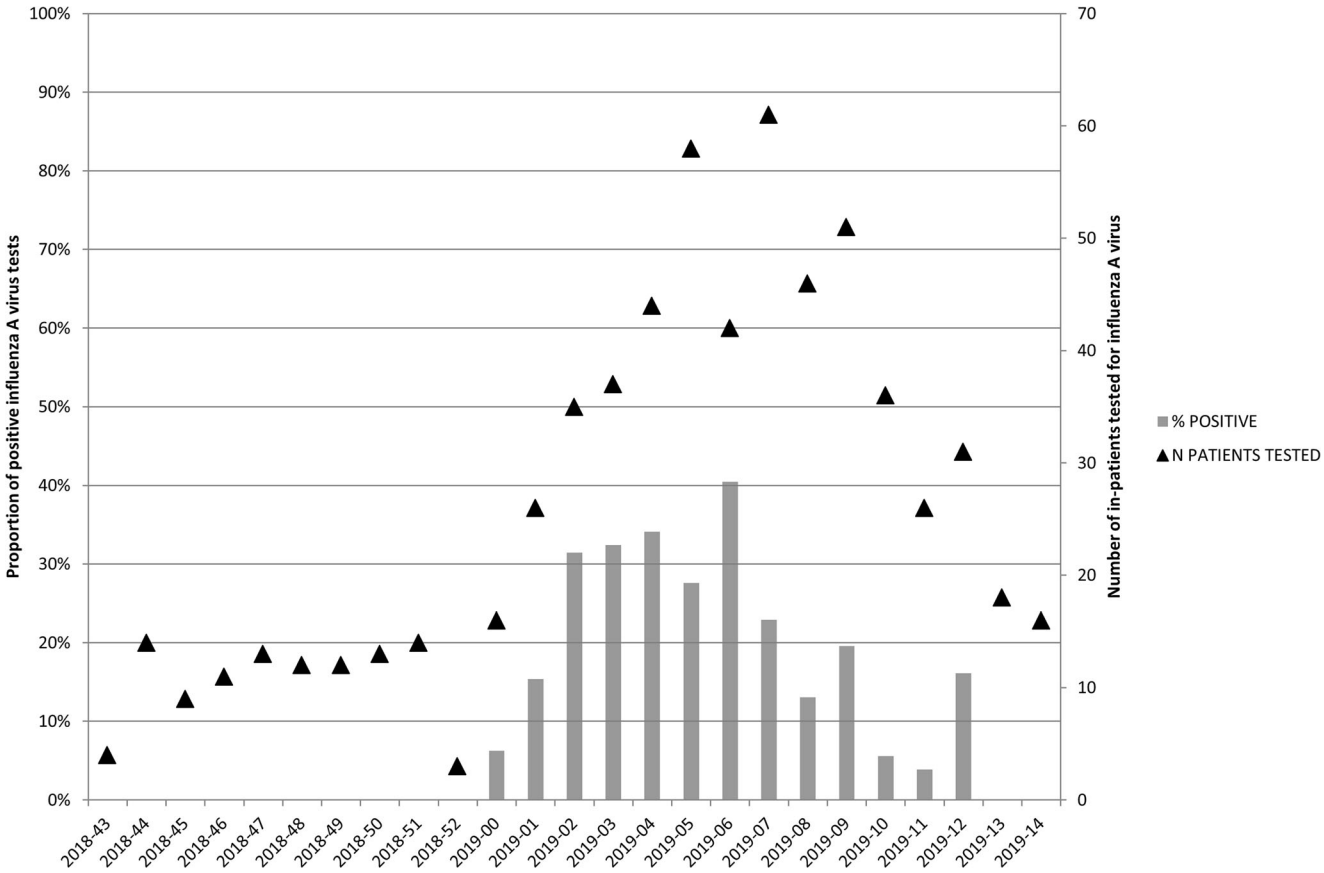

Fig. 3 Proportion of tested inpatients positive for influenza A virus by week, University Hospital of Udine, Italy, influenza season 2018–19

in Udine doubled (with a similar proportion of positivity). The arrival and availability of rapid PCR tests, which was announced and presented to all the hospital wards through official communications from the hospital management, brought attention to the issue of influenza virology diagnostics and might have induced an increase of requests, both of new rapid PCR tests and the standard ones.

Our data suggest the inappropriateness of some requests. For example, multiple requests for the same hospitalized patient were likely unnecessary. There was also evidence of tests requested on the patient's discharge date. In addition, some wards showed high numbers of test requests but low proportions of positive results, suggesting that requests were not focused. Unfocused test requests, instead of supporting clinical decisions, might paradoxically divert attention from clinical presentation, which is important for deciding whether antiviral therapy should be initiated (CDC 2019).

What is already known on this topic

Rapid PCR testing can improve the management and outcomes of hospitalized patients with respiratory illnesses. In an Australian study, for example, rapid PCR testing reduced length of stay among those who tested positive and resource utilization, regardless of positivity, and improved timeliness of care compared with standard PCR testing (Wabe et al. 2019). Our analysis cannot assess the advantages of rapid PCR tests vs. other types of testing or not testing at all, since this was an observational study with only descriptive purposes. However, the collected data were useful for organizational purposes and to identify potential sources of inappropriateness.

In Hong Kong, empirical antiviral treatment appeared to be a cost-effective strategy vs. test-guided treatment in the management of hospitalized patients with severe respiratory infection with a suspect of influenza, at least in case of influenza prevalence ≥ 2.5% (You et al. 2012). Thus, limiting urgent requests for rapid tests in patients with mild uncomplicated illness during the influenza season might be appropriate.

What this study adds

In our hospital, the highest proportions of rapid PCR test requests were among patients hospitalized in the emergency medicine ward and in the infectious diseases ward, where patients with severe or complicated illness are more likely to be found.

Requests were usually timely for patients hospitalized in medical wards (median time 1 day after admission), whereas there was greater delay in surgical wards (up to 1 week),

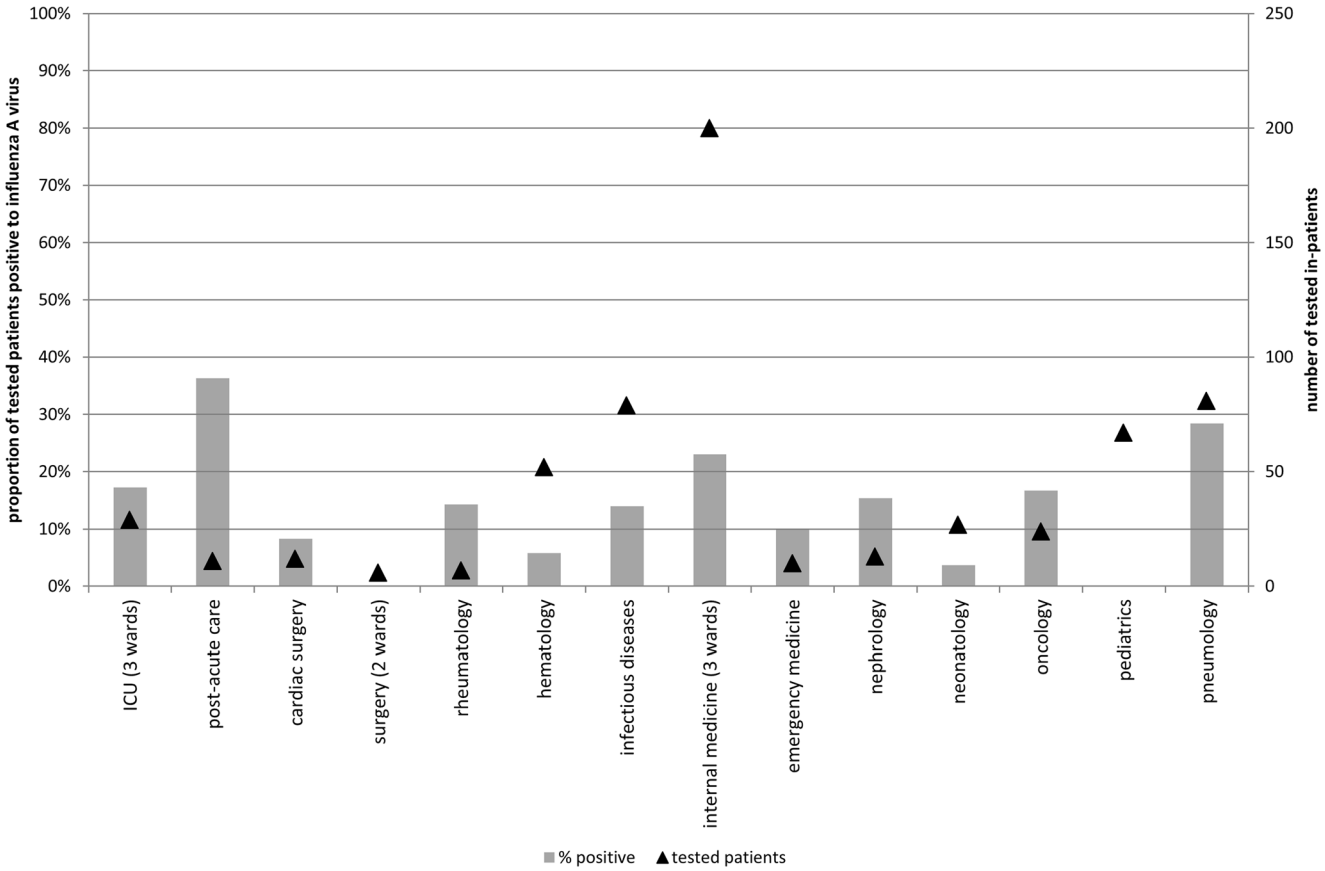

Fig. 4 Proportion of tested inpatients positive for influenza A virus by specialty of hospitalization, University Hospital of Udine, Italy, influenza season 2018–19

where cases of respiratory illness might be the result of hospital-acquired infections (Chow and Mermel 2017; Haque et al. 2018). Onco-hematologic patients were tested even later, but this is expected since these patients are not usually admitted for a respiratory infection and have high risk of hospital-acquired infection (Guinan et al. 2003).

In our hospital, patients with previous vaccination against influenza were also tested. We do not know whether the patients' vaccination status was known to the attending physicians. Anyway, the proportion of positivity among tested patients was analogous regardless of vaccination status, indicating that previous vaccination is not sufficient to rule out

Table 1 Consumption of oseltamivir doses during the influenza seasons 2017–18 and 2018–19 by medial specialty at the University Hospital of Udine, Italy

	Oseltamivir doses 2018–19	Oseltamivir doses 2017–18
ICU	188	671
Post-acute care	10	10
Cardiology	20	20
Surgery (any)	12	122
Hematology	20	30
Infectious diseases	341	240
Internal medicine	526	140
Emergency medicine	170	92
Nephrology	31	20
Oncology	70	71
Pediatrics	10	60
Pneumology	277	130
Other	140	10

influenza infection. It should be noted that our findings do not support the conclusion that vaccination was ineffective. In fact, analyzing our data according to a test-negative design would have yielded valid results only if vaccination status had not affected the probability of being hospitalized, if there was no confounding (Fukushima and Hirota 2017; Shi et al. 2017) and if every hospitalized patient with acute respiratory illness was tested (Ainslie et al. 2017), conditions that were not fulfilled in our analysis.

Direct cost of one rapid real-time PCR test was approximately 39 euros; estimated cost of one Panel 1 multiplex test, considering a 70–75% yield, was almost 12 euros. In case of unfocused requests for respiratory viruses (i.e., all 3 multiplex panels) the cost is almost 36 euros per request, very close to the cost of one rapid test. Thus, for the upcoming influenza season, we will promote requesting rapid real-time PCR tests for all cases of serious or complicated respiratory illness, limiting multiplex tests to well-defined diagnostic suspicions. The new hospital-based recommendations for requesting influenza virus tests will also have to address areas of potential inappropriateness detected in the past influenza season.

The analysis of local laboratory data, improving the knowledge and evidence base around diagnostics, provides useful information to support public health decision-making and manage diagnostic demand and supply (Engel et al. 2016). A study from six European countries showed that availability of laboratory resources may induce inappropriate demand for laboratory tests (Mrazek et al. 2020). This was at least partly true in our case, too. In agreement with the authors of that study, we believe that communication between laboratory specialists and clinicians should be intensified and improved. Educational interventions may also be useful to reduce inappropriate requests. Another strategy that could influence the clinicians' ordering behavior is the redesign of the laboratory request forms (Mrazek et al. 2020). In our hospital, we periodically revise the request forms. We must ensure that clinicians are promptly and adequately informed about those changes and that the use of the new forms is clearly explained to them.

In our study, the possibility to link laboratory data with other health-related databases allowed the integration of multifaceted information. Analysis of administrative data from the hospital health information system can be applied to other laboratory tests.

Limitations of this study

This study was descriptive so we could not assess the advantages of rapid PCR tests vs. other types of testing or no test. In addition, we did not know how the management of patients hospitalized with respiratory illness was influenced by the influenza test result and by their timeliness; nonetheless, the fact that overall consumption of oseltamivir doses during the influenza season was similar in 2018–19 and in 2017–18 suggests that the use and result of the diagnostic tests did not have a substantial impact on the consumption of antiviral drug at the hospital level. However, variations in both directions were observed in many wards, which might reflect some influence of virologic diagnoses on clinical practice.

Authorship contribution statement Francesca Valent designed the study, conducted the statistical analyses and wrote the manuscript; Francesca Malacarne contributed to data analysis and critically revised the manuscript; Sabrina Licata contributed to study design and critically revised the manuscript; Corrado Pipan contributed to study design, interpreted the results and critically revised the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval All procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Declaration of Helsinki. The analyses were based on anonymous administrative data; therefore, patient informed consent and ethics committee approval were not required in Italy. Patients were not identifiable, and their privacy rights were always observed.

Consent to participate Not applicable (the study used anonymous administrative data; patients could not be identified and no consent could be collected).

Consent to publish Not applicable (the study used anonymous administrative data; patients could not be identified and no consent could be collected).

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