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Rapid Diagnosis of Respiratory Tract Infections Using a Point-of-Care Platform Incorporating a Clinical Decision Support Algorithm

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Abstract. Respiratory Tract Infections (RTIs) are among the top reasons for visiting a General Practitioner (GP) and the main cause of unnecessary antibiotic prescriptions. Reducing inappropriate use is essential to decrease antibiotic resistance and adverse events. The goal of the Eurostars project "Respiotic" is to develop a new point-of-care (POC) platform based on the centrifugal microfluidic LabDisk that will detect the main responsible viruses and bacteria for community-acquired RTIs, including associated resistances and host biomarkers. The diagnostic platform will use a Polymerase Chain Reaction (PCR) and an immunoassay cartridge on the same instrument and provide the combined analysis within less than 1 h. An electronic clinical algorithm will co-assess the test results and act as a decision support tool for the GPs' patient management and prescriptions.

Keywords. Point-of-care diagnostics, respiratory tract infections, patient management, clinical algorithm, decision support

Introduction

Acute respiratory tract infections (RTIs) are one of the most usual causes of unnecessary antibiotic prescriptions [1], while they are also a leading cause of death. High volume of antibiotics is prescribed in ambulatory settings, mostly for acute RTIs [2]. From the clinical perspective, RTIs include a wide range of diseases from self-resolving bronchitis to potentially life-threatening bacterial or viral pneumonia. Each category requires an adapted management (from supportive care to antibiotic or antiviral treatment). From the

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epidemiologic perspective, RTIs are diseases that may emerge suddenly as seasonal epidemic (e.g. influenza) or pandemic as illustrated with the novel coronavirus SARS-CoV-2 outbreak.

The state-of-the-art in managing patients visiting their General Practitioner (GP) is via clinical examination, e.g. measuring vital signs (respiratory rate, temperature) and performing a lung auscultation. In case of pneumonia suspicion (based on clinical examination), a chest X-ray is done but its performance and availability are limited [3,4]. GPs need to manage their patients when they are still at their office, therefore, testing of collected samples in a central laboratory is difficult to integrate into this workflow. At the Emergency Departments (ED), the state-of-the-art is to collect naso-pharyngeal (NP) swabs for Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) detecting influenza and sputa for bacterial culture which may take 24-72 hours until diagnosis [4]. This long time-to-result is a major disadvantage, which may lead to unnecessary antibiotic prescription in patients with viral infection. Thus, it is evident that a rapid, accurate and easy-to-use POC tool is needed at these settings to offer a patient-side solution and support clinical decision.

From the analytical perspective, although the nucleic acid based pathogen detection is an established technology, studies have shown that for the complex syndrome of RTIs, a single diagnostic tool is not sufficient / not sensitive and specific enough, and that combining different tests can better support patient management [5]. Host biomarkers (the most studied ones being procalcitonin (PCT) and C-reactive protein (CRP)) have been used successfully to safely decrease antibiotic prescription in patients with acute RTIs but their ability to distinguish between viral and bacterial infections is limited [6]. There is an increasing clinical evidence showing the added value of the combination of microbiological tests (species identification allowing targeted antibiotic treatment) with host biomarkers (surrogate markers of etiology and severity). Therefore, it is important to perform combined tests on the same instrument. Last but not least, following the test results, the health professional needs to make a decision. Especially when the test addresses multiple factors (e.g. several RTI pathogens, biomarkers), it is not a trivial task to co-assess all results. Therefore, this requires a clinical decision support tool integrating all results for patient management [7].

In the above context, the objective of our Eurostars project “Respiotic” is to converge innovations from biotechnology, engineering and digital technologies under the umbrella of clinical expertise, in order to strengthen the healthcare professionals (GPs and ED clinicians) with a platform for: rapid detection of potential RTI viral and bacterial pathogens in combination with associated resistances and relevant biomarkers; performing all tests on the same POC instrument; combining an integrated electronic decision support algorithm for evidence-based patient management.

1. Integration of Innovative Technologies

The proposed diagnostic platform consists of the following main components (Figure 1): (i) one cartridge for detecting pathogenic species (PCR for bacteria, viruses) causing community-acquired acute RTIs and associated resistances; (ii) one cartridge for detecting host biomarkers (immunoassay for PCT); (iii) a universal instrument (Spindiag Rhonda) that can run both aforementioned cartridges either in parallel or separately, thereby: providing full integration and automation of the workflow; allowing minimum hands-on steps; offering high degree of flexibility to the healthcare professionals to

decide which cartridge(s) to use depending on the settings and the scope of their diagnosis; and (iv) an IT clinical algorithm that co-assesses the test results, together with complementary information from the clinical evaluation (selected symptoms and signs), thereby providing a holistic decision support.

Figure 1. Diagnostic workflow of the proposed POC platform. The nucleic acid cartridge detects RTI infectious agents and resistances via RT-PCR. The protein cartridge detects PCT with bead-based immunoassay. The platform integrates a clinical algorithm as decision support to enable patient management.

There are some commercial systems in the field of RTIs which offer multi-pathogen detection in an integrated way, aiming at POC or near-patient settings, indicatively the: FilmArray® (bioMérieux), Verigene® (Luminex Corp.), VerePLEX™ Biosystem (Veredus Laboratories), QIAstat-Dx (QIAGEN), Vivalytic (BOSCH), ID NOW™ (Abbott), cobas® Liat® (Roche) and the Unyvero A50 (Curetis) [8]. However, based on our market analysis, none of the technologies combines on the same instrument nucleic acid and biomarker analyses simultaneously, together with a clinical decision support algorithm, which are our major unique selling points, not only from the innovation perspective, but also from the clinical information that our combined test offers.

In the field of clinical decision support in adults with acute lower RTIs, algorithms based on host biomarkers results at primary care have been tested [9,10]. However, so far there are in literature no decision support algorithms that integrate a co-assessment of clinical signs, with host biomarkers (e.g. CRP, PCT) and the input of molecular diagnostics (i.e. genetic identification of pathogen organism and resistances), which is what we aim to do for a personalized patient management.

1.1. Microfluidic Integration of Pathogen and Biomarker Detection

The LabDisk is a centrifugal microfluidic cartridge that integrates all biochemical reagents necessary to perform a sample-to-answer analysis [11]. One half-disk cartridge will be used for nucleic acid analysis and another half-disk for immunoassay. Both cartridges are operable and compatible with a small, portable instrument (shoebox size), which has been designed to perform comprehensive diagnostics at the point of care in a user-friendly way. The cartridges are initially scanned by the scanner of the instrument and the proper method/protocol is selected. Then they are inserted in the instrument and the processing is done in a fully automated way. Each run in the Spindiag Rhonda can take two half-disks (Figure 2).

For the RTI pathogen identification, a NP swab is used for specimen collection and inserted directly into a dedicated slot of one half-disk cartridge (Figure 2), which minimizes the safety risk and allows operation even in a non-laboratory environment and

with minimum hands-on steps (for example, no pipetting is needed). The protocol then in the cartridge includes: mechanical lysis of pathogens, purification and preparation for RT-PCR, pre-amplification, main amplification after mixing with pre-stored primers/probes. The pre-amplification step allows the very high sensitivity and detection of pathogen load at very low concentrations [11]. The special heating system of the instrument allows the sample-to-answer RT-PCR results in 35 min. First priority pathogens included are *Streptococcus pneumoniae; Haemophilus influenzae* (it is the 2nd most frequent); *Mycoplasma pneumoniae* (more common in September-October); *Moraxella catarrhalis* (related for COPD exacerbation); *Staphylococcus aureus* and related resistance (*S. aureus*/mecA gene resistance); Influenza A, B, all subtypes (more common in January-February, antiviral treatment available); Respiratory Syncytial Virus (RSV) (can cause severe RTIs); SARS-CoV-2 (important public health relevance and antiviral treatment might be available in the future). The particular bacteria are chosen because they are the most common in adults with community-acquired RTI (high pre-test probability). Other Gram-negative bacteria included in commercial systems (e.g. *Pseudomonas aeruginosa*, *Enterobacter cloacae*) are identified in patients with nosocomial RTIs. Testing for these pathogens in the wrong target patients population (low pre-test probability) increases the risk of mis-interpretation of the results (high number of false positive results: detection of colonization rather than infection). Thus, they are not included in our study. The viruses are chosen because they can cause a severe infection, there is an antiviral treatment or they are of public health relevance. If needed, the panel is easily adaptable and expandable to cover up to 24 pathogens. In case a presumably sick patient is found negative for all pathogens, then the decision to prescribe antibiotics or not will be based on PCT, which is a major advantage of the platform.

For biomarker detection, blood is collected by the health professional and inserted into the second half-disk cartridge, which also, like the PCR-cartridge, integrates onboard all necessary biochemical reagents, in this case micro/nanoparticles and one assay buffer for the assay realization. The workflow starts with a plasma separation module, and continues with the assay itself, which is based on rapid (15-20 min), single-step bead-based immunoassay.

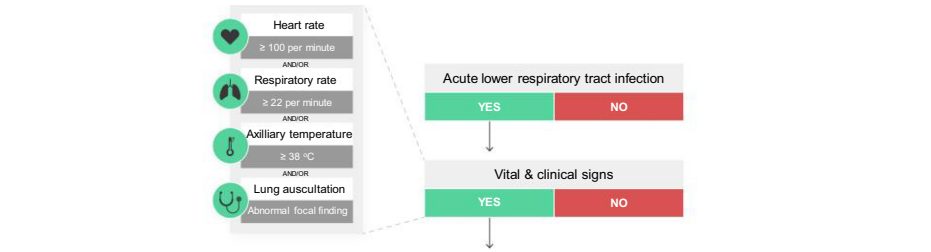
Figure 2. Left: A swab is inserted into the LabDisk PCR-cartridge. Right: The cartridge is inserted into the compact, portable Spindiag Rhonda instrument (20 x 40 x 28 cm).

1.2. Clinical Algorithm as Decision Support

The clinical algorithm is developed based on national, international guidelines, and a review of the literature on the performance of clinical predictors, the accuracy of laboratory tests and the first-line treatment according to epidemiological data on antibiotic resistance levels [4]. It will integrate optimal clinical parameters and POC diagnostic tests (RT-PCR and biomarkers concentrations). The algorithm will be tested in a prospective cohort of patients with acute lower RTIs included in GP practices [12].

Between September 2018 and March 2020, 450 patients were prospectively included and samples were collected (plasma and NP swabs) in 60 Swiss GP practices. Using biobanked samples, PCT will be measured and multi-target RT-PCR will be performed. Then, the algorithm will be adapted based on statistical analyses of these real-life data to get the best performance in terms of sensitivity, specificity, positive and negative predictive values. It will also integrate guidance on the necessity to (i) run the PCR-biomarker test (diagnostic stewardship); and (ii) give an antimicrobial treatment (antimicrobial stewardship). In future prospective clinical trial (validation cohort), the performance and safety of the algorithm will be tested.

The next step is the digitalization of the algorithm and the development of the mobile application (eRespi) using an open-source schema structure. We developed a user-friendly software which will allow flexibility and adaptability for different contents such as questions, images, videos and interactive charts. All algorithms are then entered into the system as digital e-algorithms. The mobile application eRespi is developed to provide the clinicians with an intuitive interface for interacting with the e-algorithms. The application can function offline and allows managing several patients in parallel (pausing and resuming the patient file). According to GP preference, the application can be used on a tablet or directly on his/her computer. Vital signs (blood pressure, pulse rate, respiratory rate and oxygen saturation) can be measured and automatically connected to the GP computer (or tablet). The RT-PCR and biomarker analysis results from the LabDisks will also feed into this workflow. A report will be generated at the end of the session for review by the clinician, containing a recommendation for patient management (Figure 3 for version v1.0 of the eRespi, which will be continuously updated as real-life cohort data will be analyzed and more pathogens will be inserted in the LabDisk panel).



		Point of Care									
		Multiplex PCR									
		INFLUENZA	MYCOPLASMA PNEUMONIAE	STREPTOCOCCUS PNEUMONIAE	HAEMOPHILUS INFLUENZAE	MORAXELLA CATARRHALIS	STAPHYLOCOCCUS AUREUS		Respiratory Syncytial Virus	SARS-CoV-2	NEGATIVE
							Methicillin resistant	Methicillin sensitive			
Procalcitonin [µg/L]	≥ 0.25 At	AMOXICILLIN CLAVULANATE + NEURAMINIDASE INHIBITOR*	CLARITHRO-MYCIN	AMOXICILLIN	AMOXICILLIN CLAVULANATE	AMOXICILLIN CLAVULANATE	LINEZOLID	AMOXICILLIN CLAVULANATE	AMOXICILLIN CLAVULANATE	AMOXICILLIN CLAVULANATE + COVID-19 TREATMENT	AMOXICILLIN CLAVULANATE
	< 0.25	NEURAMINIDASE INHIBITOR*	CLARITHRO-MYCIN	NO ANTIBIOTICS	NO ANTIBIOTICS	NO ANTIBIOTICS	NO ANTIBIOTICS	NO ANTIBIOTICS	NO ANTIBIOTICS	COVID-19 TREATMENT	NO ANTIBIOTICS

*for patient at high risk of complication

Figure 3. Indicative e-algorithm integrating clinical symptoms and signs, PCR and biomarkers results in a decision support management tool. In case one of the four clinical signs is positive, a POC test is required.

2. Expected Output and Impact

The expected output of the "Respiotic" project lies in innovative technological breakthroughs such as: (i) the combination of nucleic acid and protein biomarker analysis

simultaneously and on the same instrument, providing utmost flexibility to the end-user; (ii) the combination of rapid and multiple target diagnostic POC system; (iii) the combination of this molecular diagnostic tool with a clinical algorithm for data co-assessment from various sources, for clinical decision support that will enable the GP/clinician to perform the test immediately, and rationally prescribe antibiotics or antivirals, only when the condition requires. Such platform will guide the clinicians in the management of their patients and integrate diagnostic and antimicrobial stewardship. The project impact is expected at multiple levels: (i) shifting the diagnostic methodology from empirical or traditional laboratory approach towards molecular analysis that will accelerate and improve patients management; (ii) providing more accurate diagnosis, reducing the inappropriate use of antibiotics; (iii) decentralizing diagnosis and strengthening the front-line diagnostic landscape, thereby offering efficient management of patients at the point of need and relieving the burden of hospitals and clinics.

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References

[1] Hurlimann D, Limacher A, Schabel M, et al. Improvement of antibiotic prescription in outpatient care: a cluster-randomized intervention study using a sentinel surveillance network of physicians. J Antimicrob Chemother. 2015; 70,2:602-8.
[2] Schappert SM, Burt CW. Ambulatory care visits to physician offices, hospital outpatient departments, and emergency departments: United States, 2001-02. Vital Health Stat. 2006; 13,159:1-66.
[3] Ye X, Xiao H, Chen B, Zhang S. Accuracy of lung ultrasonography versus chest radiography for the diagnosis of adult community-acquired pneumonia: review of the literature and meta-analysis. PLoS One. 2015; 10,6:e0130066.
[4] Woodhead M, Blasi F, Ewig S, et al. Guidelines for the management of adult lower respiratory tract infections-full version. Clin Microbiol Infect. 2011; Suppl 6:E1-59.
[5] Wunderink RG, Waterer G. Advances in the causes and management of community acquired pneumonia in adults. BMJ. 2017; 358:j2471.
[6] Self WH, Balk RA, Grijalva CG, et al. Procalcitonin as a Marker of Etiology in Adults Hospitalized With Community-Acquired Pneumonia. Clin Infect Dis. 2017; 65,2:183-90.
[7] van Belkum A, Bachmann TT, Lüdke G, et al. Developmental roadmap for antimicrobial susceptibility testing systems. Nat Rev Microbiol. 2019; 17:51-62.
[8] Mitsakakis K, D’Acremont V, Hin S, et al. Diagnostic tools for tackling febrile illness and enhancing patient management. Microelectron Eng. 2018, 201:26-59.
[9] Burkhardt O, Ewig S, Haagen U, et al. Procalcitonin guidance and reduction of antibiotic use in acute respiratory tract infection. Eur Respir J. 2010; 36(3):601-7.
[10] Briel M, Schuetz P, Mueller B, et al. Procalcitonin-guided antibiotic use vs a standard approach for acute respiratory tract infections in primary care. Arch Intern Med. 2008; 168,18:2000-7.
[11] Czilwik G, Messinger T, Strohmeier O, et al. Rapid and fully automated bacterial pathogen detection on a centrifugal-microfluidic LabDisk using highly sensitive nested PCR with integrated sample preparation. Lab Chip. 2015; 15,18:3749-59.
[12] Lhopitallier L, Kronenberg A, Meuwly JY, et al. Procalcitonin and lung ultrasonography point-of-care testing to decide on antibiotic prescription in patients with lower respiratory tract infection in primary care: protocol of a pragmatic cluster randomized trial. BMC Pulm Med. 2019; 19:143.