

# The Netherlands Journal of Medicine

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*A fatal case of thrombocytopenia, kidney failure and hemolysis; what is your diagnosis?*

VENOUS THROMBOSIS DURING OLANZAPINE TREATMENT

TREATMENT OUTCOMES IN IGG4-RELATED DISEASE

SYNDROMIC SAMPLE-TO-RESULT PCR TESTING

HYDROXYCARBAMIDE INDUCED PNEUMONITIS

SUDDEN SUBCUTANEOUS EMPHYSEMA AND DYSPHAGIA

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# Medication and venous thromboembolism: a complex interaction

S. Schol-Gelok<sup>1\*</sup>, M.J.H.A. Kruip<sup>2</sup>, J. Versmissen<sup>1</sup>

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In the pathogenesis of venous thromboembolism (VTE) three main components were identified by Virchow, a nineteenth century German physician: alterations in blood coagulation, diminished blood flow or damage of the vascular endothelium, or a combination of these factors.<sup>1</sup> Risk factors of VTE such as immobility, active infection or cancer, pregnancy, trauma, advanced age, antiphospholipid antibodies, obesity and certain genetic traits such as the factor V Leiden mutation all influence one or more of these three components.<sup>2</sup> Additionally, it has gradually become clear that many drugs can lower or increase the risk of VTE through different mechanisms influencing the triad of Virchow.

Antiplatelet drugs, such as aspirin and clopidogrel, inhibit thrombocyte aggregation and decrease thrombus formation. As expected, they reduce the risk of VTE and have been considered as secondary prevention in patients with VTE.<sup>3</sup> Other, less expected groups of drugs may also lower the risk of thrombosis. HMG-CoA reductase inhibitors for example, more commonly known as statins, lead to a lower risk of venous thrombosis, as confirmed in a recent meta-analysis of intervention studies: the risk of a primary venous thrombosis was 15% lower in the statin-treated group.<sup>4</sup> This is probably due to inhibition of geranylgeranylation of the Rho/Rho kinase pathway as one of the key mechanisms of the anticoagulant effects.<sup>5,6</sup> During the 1990s it became strikingly obvious that certain drugs could also increase the risk of VTE. Based on several case series describing an association between oral contraceptives and a higher risk of VTE, a large case-control study was eventually performed by the World Health Organization (WHO). This study confirmed a two- to four-fold increase of the risk of VTE in oral contraceptive users, particularly with third generation contraceptives.<sup>7</sup> A riot ensued when both the German Federal Institute for drugs and medical services and the British government initially discouraged the use of third generation oral contraceptives because of this

increased risk of VTE. The European Medicines Agency (EMA) and Food and Drug Administration (FDA), on the other hand, had decided that these drugs should not be withdrawn. This resulted in many more studies, which were evaluated in a Cochrane review in 2014, finally concluding that oral contraceptive users did indeed run a higher risk of VTE, while the risk third generation users ran was only slightly higher than that of second generation users.<sup>8</sup> Glucocorticoids, another class of commonly prescribed drugs, are also well known for their increased risk of thrombosis, as expected by their working mechanism, which leads to increased levels of clotting factors and fibrinogen.<sup>9</sup> Based on their working mechanism, other, less frequently prescribed medications are also expected to increase the risk of VTE. For example, anti-epidermal growth factor receptor (EGFR) agents, classified as either monoclonal antibodies (MoAbs) or tyrosine kinase inhibitors (TKIs) are both associated with a significant increase of the risk of VTE.<sup>10</sup> The most difficult associations to detect are in the groups of drugs that unexpectedly increase the risk of VTE. In this issue of the *NJM* Dijkstra and Van der Weiden et al. describe a case of a schizophrenic patient who was diagnosed with a deep venous thrombosis six months after starting olanzapine. The authors found disproportionate Reporting Odds Ratios (RORs) in the global database for adverse drug reactions for VTE and olanzapine. The mechanism behind this association seems to be multifactorial, with lethargy and weight gain after starting olanzapine treatment being the most likely risk factors in the development of VTE.

The ROR has been developed as a hypothesis generating tool for the detection of signals of an association between a certain drug and a side effect.<sup>11</sup> It is based on spontaneous reporting from various resources to the pharmacovigilance databases such as the Netherlands Pharmacovigilance Centre of Lareb and the worldwide Vigilyze pharmacovigilance database maintained by the WHO collaborating centre for international drug monitoring. As shown by

the publication of several case series about the association between oral contraceptives and the higher risk of VTE, it remains vital that physicians keep reporting to pharmacovigilance databases any unexpected case of VTE that might be related to a certain drug. This will increase our knowledge of the risk of thrombosis and possibly prevent new events.

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# Venous thrombosis during olanzapine treatment: a complex association

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## ABSTRACT

Olanzapine, a second generation antipsychotic, has previously been associated with an increased risk of venous thromboembolism (VTE).

In this mini-review we describe a case of a thirty-year-old schizophrenic patient who was diagnosed with a deep venous thrombosis (DVT) six months after starting olanzapine therapy, as well as seventeen other VTE cases in patients using olanzapine reported to the Netherlands Pharmacovigilance Centre Lareb. In 14 of these reports, patients had reported additional risk factors for VTE.

We found disproportionate Reporting Odds Ratios (RORs) in the global database VigiBase for olanzapine and the reactions deep vein thrombosis (ROR of 1.38 with a 95% CI (Confidence Interval) of 1.22-1.57) and pulmonary embolism (ROR of 1.99 with a 95% CI of 1.81-2.19).

The mechanism behind the association of olanzapine with VTE could be explained by two risk factors, substantial weight gain and lethargy, both common side effects of olanzapine. So far, a direct effect of olanzapine on platelet aggregation or coagulation has not been found.

Schizophrenic patients are more likely to have diagnostic delay in the diagnosis of VTE, as symptoms such as lethargy and impaired pain perception result in diminished pain perception and pain expression, while they are at increased risk of developing VTE.

Currently no validated risk score is available for detection of psychiatric patients who might benefit from pharmacologic VTE prophylaxis. In patients developing a VTE while being treated with olanzapine, discontinuation of olanzapine could be considered based on the

individual risk profile, control of psychotic symptoms and antipsychotic treatment options.

## KEYWORDS

Antipsychotic agents, deep venous thrombosis (DVT), olanzapine, pulmonary embolism (PE), risk factors

## INTRODUCTION

Olanzapine is a second generation antipsychotic (SGA) commonly prescribed for treatment of positive symptoms of schizophrenic patients. Common side effects of SGAs include diabetes, metabolic syndrome, sexual dysfunction, hyperprolactinemia and weight gain, the latter more frequently seen in olanzapine.<sup>1</sup> A less known adverse event is that the use of olanzapine and other SGAs increase the risk of venous thromboembolism (VTE).<sup>1,2</sup>

VTE is a multifactorial disease with broadly two presenting entities: deep venous thrombosis (DVT) or pulmonary embolism (PE). Risk factors of VTE include immobility, sedation, previous VTE, active disease (e.g. infection or cancer), smoking, trauma, advanced age, male gender, hyperprolactinemia, antiphospholipid antibodies, obesity and certain genetic traits such as the factor V Leiden mutation.<sup>2</sup>

Both the Dutch website for drug information 'Farmacotherapeutisch Kompas' and the Dutch Summary of Product Characteristics (SmPC) of olanzapine mention

VTE as an uncommon (0.1-1%) adverse drug reaction.<sup>3,4</sup> The SmPC of olanzapine describes no causal relationship has been established and that patients with schizophrenia often have acquired risk factors for VTE. They recommend identifying all possible risk factors for VTE and taking preventative measures.

The annual incidence of VTE is 1 per 1000 in adult populations.<sup>5</sup> The risk of developing VTE is increased by 2.20 (odds ratio (OR), 1.22-3.95 (95% confidence interval (CI))) in patients using SGAs.<sup>6</sup>

We describe a case of a young man with a history of paranoid schizophrenia who presented with a DVT in our hospital (the Maasstad Hospital in Rotterdam, the Netherlands) while using olanzapine. By appraising the reported cases in the databases of the Netherlands Pharmacovigilance Centre Lareb and VigiBase (the worldwide pharmacovigilance database maintained by the WHO collaborating centre for international drug monitoring UMC (Uppsala Monitoring Centre in Sweden)), we further explored the possible association between olanzapine and VTE. Lastly we performed a brief literature review on the potential mechanisms behind this association.

## CASE REPORT

A thirty-year-old man was diagnosed with a DVT in our hospital in March 2016, six months after initiating olanzapine treatment. He was admitted to a long-term psychiatric ward for treatment of his therapy resistant paranoid schizophrenia and comorbid cocaine and amphetamines addiction.

In July 2015, he suffered from continuous hallucinations and delusions despite having been treated with several antipsychotics. The Dutch multidisciplinary guideline for Schizophrenia advises clozapine treatment when two different types of antipsychotics are not effective.<sup>7</sup> However, our patient preferred olanzapine treatment and in August 2015 he was started on 300 mg olanzapine dosed by intramuscular injection once every four weeks.

By February 2016, he had gained 22 kg in weight (Body Mass Index (BMI) increase from 22.6 to 28.9) and had adopted a sedentary lifestyle, spending most of the day in bed. His high-density-lipoprotein (HDL) was reduced (0.83 mmol/l, reference level > 1 mmol/l) and his waist circumference had increased from 90 to 117 cm (reference < 102 cm). His blood pressure, glucose and triglyceride levels were normal. As only two out of five criteria obtained he did not qualify for metabolic syndrome.<sup>8</sup>

In March 2016, he presented at our hospital with a red swollen tender lower right leg. Ultrasound confirmed the diagnosis of DVT and the patient was started on

anticoagulant treatment with a vitamin K antagonist with low molecular weight heparin injections until the desired prothrombin times were achieved.

Risk factors for DVT in this patient were male gender, active psychosis, use of olanzapine, lethargy and high BMI after his significant and ongoing weight gain. Persisting psychotic symptoms and the presence of DVT made us decide to discontinue olanzapine and start clozapine. Within the next few months our patient became more active and less psychotic, however his BMI increased further to 31 kg/m<sup>2</sup>. Antithrombotic treatment was discontinued after three months, following the Dutch guidelines prevalent at that time.<sup>9</sup>

## DISCUSSION

### Other reports from Lareb and VigiBase

From 2001 to the 26<sup>th</sup> of January 2018 Lareb received 18 reports of DVT or PE associated with the use of olanzapine, including the previously discussed case report, see *table 1* for further details.<sup>10</sup> In 14 of these reports, patients had additional risk factors for VTE.

VigiBase lists 241 reports on olanzapine (with a Reporting Odds Ratio (ROR) of 1.38 [95% CI 1.22-1.57]) of the reaction “deep venous thrombosis” (DVT) and 441 (ROR 1.99 [95% CI 1.81-2.19]) reports of the reaction “pulmonary embolism”(PE), including the cases received by Lareb.<sup>11</sup> The ROR is a measure of disproportional reporting in the database, and in this case a significantly increased ROR indicates that DVT or PE is more often reported for olanzapine use than with other drugs in the database. The ROR has been developed as a method for signal detection; it is therefore a hypothesis generating tool.<sup>12</sup> The only other antipsychotic with a significantly increased ROR for these reactions was clozapine, with an ROR of 1.15 [95% CI 1.14-1.37, 473 reports] for DVT and an ROR of 2.15 [95% CI 2.02-2.29, 1024 reports) for PE. The RORs from VigiBase for various antipsychotics are described in detail in *table 2*.

Among the cases reported on in Lareb and VigiBase, the likelihood of a causal association between a drug and a reaction may vary as both are based on spontaneous reporting from various sources with different degrees of documentation.

### Cases in the scientific literature

Various case reports in the scientific literature describe the association of olanzapine with VTE.<sup>13-16</sup> Most case reports describe male patients aged sixty years and older, although PE has also been described in a 28-year-old male patient shortly after starting olanzapine treatment.<sup>16</sup> Only a few population studies have reported on olanzapine

**Table 1.** Reports received by the Netherlands Pharmacovigilance Centre Lareb of deep venous thrombosis or pulmonary embolism associated with the use of olanzapine<sup>8</sup>

Report details	Number of reports
Gender	Male (13), female (5)
Age	Range: 26-71 years old, median 45 years old, not reported in one case
Indications for olanzapine	Schizophrenia (6), psychosis (5), bipolar disorder/mania (4), not reported (3)
Reported VTE (venous thromboembolism):	Deep vein thrombosis (6*), pulmonary embolism (12)
Latencies (time from starting olanzapine till event)	2 days – 13 years, median 5 months
Discontinuation of olanzapine	Continuation in same dose (5), continuation in reduced dose (3), discontinuation (5), unknown (5)
Follow-up	Recovery/recovering of VTE (12), no recovery at time of reporting (1), unknown (3), death of any cause with uncertain possible relationship with drug (2)
Pharmacologic treatment of VTE	Yes (15), unknown (3)
Concurrent risk factors	Possible risk factors reported (14): factor V Leiden (1), smoking (2), other antipsychotic drugs or oral contraception associated with thromboembolism as concomitant medication (4), immobilization (3), significant weight increase (5), reported Body Mass Index > 30 kg/m <sup>2</sup> (2), family history with thromboembolism (1), significant comorbidity (2)

\*In one of these reports the patient also experienced occlusion in an artery

**Table 2.** Reporting Odds Ratios (ROR) from VigiBase

Antipsychotics	ROR for deep vein thrombosis		ROR for pulmonary embolism	
	ROR (95% CI)	N	ROR (95% CI)	N
Olanzapine	1.38 (1.22-1.57)	241	1.99 (1.81-2.19)	441
Clozapine	1.25 (1.14-1.37)	437	2.15 (2.02-2.29)	1024
Quetiapine	0.65 (0.55-0.77)	141	0.97 (0.86-1.10)	266
Risperidone	0.66 (0.57-0.77)	184	1.08 (0.98-1.20)	381
Sertindole		0	4.19 (2.24-7.83)	10
Paliperidone	0.47 (0.35-0.62)	48	0.76 (0.63-0.93)	99
Aripiprazole	0.48 (0.38-0.60)	70	0.81 (0.69-0.95)	151
Lurasidone	0.35 (0.13-0.94)	4	0.83 (0.47-1.47)	12

specifically.<sup>6</sup> A 2014 meta-analysis reports an OR of 1.35 (95% CI 0.97-1.89,  $p = 0.08$ ) for the risk of VTE in patients using olanzapine. The results of this meta-analysis should be interpreted with caution since the quality and inclusion criteria varied between studies leading to between-study heterogeneity. This resulted in a low statistical power and a wide and nonsignificant confidence interval for all included SGAs.<sup>17</sup> On the other hand a large case control study reported a significant OR for risk of VTE in patients using olanzapine of 1.49 (95% CI 1.07-2.08).<sup>18</sup>

Risk of VTE appears to be especially elevated in the first months of SGA treatment (OR 1.97; 95% CI 1.66-2.33). For users of antipsychotic drugs the risk was 56% higher compared to non-users (OR 1.56; 95% CI 1.39-1.75).<sup>19</sup> However, current data can neither conclusively verify differences in occurrence rates of VTE between first- and second-generation antipsychotics nor identify which antipsychotic drugs have the lowest risk of VTE, though one might speculate that the risk of VTE is higher for clozapine than for other SGAs.<sup>20</sup>



### Potential mechanism

The potential underlying mechanism explaining the higher risk of VTE during antipsychotic treatment is not yet fully clear. Various factors seem to play a role, especially metabolic syndrome, a common side effect of olanzapine treatment as 34% of schizophrenic patients taking olanzapine monotherapy fulfil its criteria.<sup>21</sup> Metabolic syndrome is a known risk factor of VTE.<sup>22</sup>

Immobilisation, a consequence of the lethargy caused by various antipsychotics, is linked to increased risk of VTE due to venous stasis and blood pooling in the lower extremities. Obesity is also an independent risk factor for VTE and although all SGAs are associated with some weight gain and increased appetite, olanzapine and clozapine have the most profound impact compared to non-SGA antipsychotics and placebo.<sup>23-25</sup> A database analysis comprising 3507 patients in 21 placebo- and active-controlled studies conducted in America, Australia, New Zealand and Europe showed that 48% of patients taking olanzapine experience > 7% weight gain within the first 12 weeks, and 57% of patients experience a significant weight gain within the first 6-12 months with a median weight gain of 0.7 kg per month, compared to placebo (incidence of weight gain in placebo was 13%).<sup>24</sup> This increase in body weight of at least 20% is more pronounced in inexperienced users of antipsychotics.<sup>25</sup> Only in olanzapine users a significant increase in weight was found when comparing the weight at > 38 weeks to the weight at six weeks after starting olanzapine.<sup>25</sup> Therefore, if a patient is already on olanzapine, switching to a different antipsychotic drug, such as haloperidol, might be indicated.<sup>26</sup> In one case, switching from olanzapine to aripiprazole resulted in 6.6% weight loss without further impairment of psychological functioning.<sup>27</sup>

There is less information available regarding the presence of lethargy in schizophrenic patients treated with antipsychotics. It is difficult to compare trials as they do not always give a clear description of somnolence, sedation, lethargy and hypersomnia. Nevertheless olanzapine, quetiapine, risperidone and especially clozapine are all associated with significantly more of these symptoms compared to placebo.<sup>28</sup> About 25-39% of patients taking olanzapine experience sleepiness, which is significantly more than placebo (26.2% compared to 15.3%), whereas somnolence is experienced by 26-46% of patients taking clozapine.<sup>28,29</sup> It has been postulated that in antipsychotic-induced somnolence blockade of histamine 1 receptors and  $\alpha_1$  receptors play an important role.<sup>30,31</sup>

Other associated risk factors include raised levels of antiphospholipid antibodies and hyperprolactinemia. The exact roles of these risk factors in a clinical psychiatric setting still need to be determined.<sup>32</sup>

It is hypothesized that antipsychotics directly influence the risk of VTE, in particular second generation antipsychotics such as clozapine and olanzapine. Many antipsychotics antagonise the serotonin (5-HT<sub>2A</sub>) receptors. As these receptors are also present on platelets, the antipsychotic medication might influence platelet aggregation.<sup>33</sup> Paradoxically, most studies investigating this mechanism suggested a lower risk of VTE.

Almuqdad et al. have shown that risperidone, and not olanzapine, leads to a clinically significant inhibition of platelet aggregation induced by serotonin. When adding these antipsychotics to a serotonin-epinephrine combination, both weak platelet agonists affecting respectively the 5-HT and the  $\alpha_2A$ -adenergic receptors on platelets, a dose-dependent inhibition of platelet aggregation was found. However, no statistically significant inhibitory effect on platelet aggregation was seen with olanzapine, possibly due to a lower 5-HT<sub>2A</sub> and  $\alpha_2A$ -adenergic receptor affinity compared to e.g. risperidone.<sup>31</sup> Another in vitro study indicates that clozapine and olanzapine show a strong inhibitory effect on ADP-stimulated platelet aggregation, which would also lower the risk of VTE.<sup>34</sup> Yet another in vitro study has shown increased platelet adhesion and aggregation for clozapine, but not for olanzapine.<sup>32</sup>

These results suggest that the serotonin receptor effect does not account for the increased risk of VTE while using SGA. In absence of a clear pathogenic mechanism to account for the associated higher risk of VTE in olanzapine users, we might conclude that this association is most probably caused by risk factors like substantial weight gain and lethargy, not by olanzapine itself.

### Diagnosing VTE in psychiatric patients

VTE is known to have a diagnostic delay, the average delay in diagnosing PE is 8.6 days, where patient delay is on average 4.2 days and delay in primary care is 3.9 days on average.<sup>35</sup> 23.8% of patients are diagnosed at least a week after onset of symptoms. The absence of chest symptoms is associated with a diagnostic delay with an OR of 5.4 (95% CI 1.9-15).<sup>36</sup> Specifically, patients with a diagnostic delay were less likely to present with chest pain (24% vs 54%,  $p = 0.003$ ) or pain during inspiration (9% vs 33%,  $p = 0.011$ ) compared to patients without diagnostic delay. Pain perception in schizophrenic patients is impaired in various ways, without an exact mechanism being clear.<sup>37</sup> Also, cognitive impairment and excess negative symptoms influence the expression of pain in this patient category.<sup>38</sup> For example, in a case report of a 75-year-old patient diagnosed with catatonic schizophrenia and with PE the patient could not express any pain symptoms, which stresses the fact that schizophrenic patients are more

likely to have diagnostic delay in the diagnosis of VTE.<sup>39</sup> As venous thromboembolism is an important cause of mortality and morbidity it is important to take this into account. An observational study including systematic venous ultrasound identified DVT in 10 out of 449 patients (2.2%) after 10 days admission to a psychiatric ward. Within 90 days 17 patients developed VTE including three symptomatic PEs.<sup>40</sup>

### Options for prophylactic treatment

Identifying patients on antipsychotic therapy who have a high risk of developing a VTE might be difficult as no validated risk score is available that is able to detect which psychiatric patients might benefit from pharmacologic VTE prophylaxis. For olanzapine use alone, an OR of 1.35 for the risk of venous thrombosis does not seem to justify prophylactic treatment with low molecular weight heparins (LMWH). However, additional risk factors for VTE have been identified in psychiatric patients.

One risk score designed specifically for psychiatric patients includes the use of antipsychotics.<sup>41</sup> Other risk factors included are history of VTE, cancer (active/treated), age, acute infectious/respiratory disease, immobilization (including catatonia), hormone therapy, obesity (BMI > 30), dehydration and thrombophilia. Based on the presence of these risk factors patients could be categorized according to their low, medium or high risk of developing a DVT with the recommendation to start (LMWH in the medium and high risk populations. However, this risk score and the algorithm derived from it have not yet been validated in a larger setting and it is unclear whether the benefits of the prophylactic treatment with anticoagulants (e.g. LMWH) outweigh possible side effects, for example an increased risk of bleeding.

The Padua risk score has been validated in a larger clinical setting of medical inpatients and has been proven to distinguish between inpatients with high risk of VTE and patients with low risk. The risk factors involved are active malignancy, previous VTE, immobility, thrombophilia, trauma and/or surgery less than one month ago, age over 70 years, cardiovascular and or respiratory diseases, acute infection and or rheumatologic abnormalities, a BMI of more than 30 and hormonal therapy.<sup>42</sup>

Nevertheless, this model was not specifically tested in psychiatric patients and only in an acute hospital setting.<sup>42</sup> Therefore we also cannot use the Padua risk score in a psychiatric ward for deciding on whether to give pharmacological prophylactic treatment.

## CONCLUSION

SGAs such as olanzapine are commonly prescribed for treatment of positive symptoms of schizophrenia, but are associated with a higher risk of VTE. This association is most likely explained by associated risk factors such as substantial weight gain and lethargy, both side effects of olanzapine. Further studies are required to determine the potential mechanisms of olanzapine on for example thrombogenicity and platelet aggregation.

In general, it is important to realise that diagnosing VTE in schizophrenic patients can be more difficult, due to symptoms such as lethargy and impaired pain perception. No validated risk score is available for detection of psychiatric patients who might benefit from pharmacological VTE prophylaxis. In patients who develop VTE while being treated with a SGA such as olanzapine, discontinuation of olanzapine might be considered based on individual risk profile, control of psychotic symptoms and antipsychotic treatment options.

## DISCLAIMER

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## DISCLOSURES

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# Mycobacterial skin and soft tissue infections: TB or not TB?

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## ABSTRACT

Non-tuberculous mycobacteria are a known cause of skin and soft tissue infections. However, only too often it takes inordinately long to arrive at the appropriate diagnosis and start treatment. Actively searching for predilection factors, exposure risks and specific clinical clues may speed up the diagnostic process. Deep tissue biopsy cultures are indispensable to determine the species and strain of mycobacterium, with important consequences for treatment. Less well known as a causative agent of prolonged tenosynovitis is *Mycobacterium tuberculosis*. We present a case series and performed a literature search concerning mycobacterial tenosynovitis.

## KEYWORDS

Tuberculous tenosynovitis, musculoskeletal tuberculosis, chronic tenosynovitis

## INTRODUCTION

Chronic tenosynovitis is known as one of the clinical presentations of nontuberculous mycobacterial infection, but is rarely described as the sole presenting symptom of *Mycobacterium tuberculosis* infection or tuberculosis. In 15-30% of tuberculosis cases, one or multiple extra-pulmonary manifestations are noted.<sup>1</sup> In this group, musculoskeletal involvement is seen in 10-15%, most often as spinal tuberculosis or Pott's disease.<sup>2</sup> Tuberculous tenosynovitis is a rare presentation of musculoskeletal tuberculosis, mostly described in immunocompetent patients.<sup>1,3</sup> In contrast, nontuberculous mycobacteria (*M. marinum*, *M. abscessus*, *M. chelonae* and

### What was known on this topic:

Chronic tenosynovitis can be caused by mycobacteria. Depending on the microorganism identified, antibiotic treatment differs.

### What does this add:

Not only non-tuberculous, but also tuberculous mycobacteria may cause chronic tenosynovitis. Deep tissue cultures are necessary to correctly identify the causative microorganism.

other species) cause skin and soft tissue infections more frequently. They are ubiquitous environmental organisms, acquired by direct inoculation. There is no evidence for animal-to-human or human-to-human transmission.<sup>4</sup> Four cases of mycobacterial skin and soft tissue infections diagnosed between 2013 and 2015, including one case involving *M. tuberculosis*, show how important lessons can be drawn from the observed delay in diagnosis, ranging from the importance of clinical clues pointing towards mycobacterial involvement to the appropriateness of tissue cultures and biopsy specimens. To broaden the perspective on these rarer clinical phenomena a literature search on mycobacterial tenosynovitis was also performed.

## CASE SERIES

### Case 1

A 75-year-old woman presented with painful swelling of the wrist, expanding to the little finger. Despite multiple investigations and both medical and surgical treatments (table 1), no definite diagnosis or cure could be established after 12 months. Differential diagnoses included carpal tunnel syndrome and presence of a pseudocyst. Final

**Table 1.** Schematic overview of characteristics of the individual cases in the presented case series

Gender/age	Initial trauma	Symptoms	Diagnostic delay	Immuno-suppression	Exposure to NTM/TBC	Imaging	Histological findings	Bacteriological findings	Surgical treatment	Antibiotic treatment
F/75	Recalls nothing	Swelling of wrist and later little finger, stiffness	12 months	None	Gardening, cleaning with water outdoors	Ultrasound: collection skeletal scintigraphy: diffuse increased uptake NMR: tenosynovitis	1. Granulomas 2. Granulomas 3. Macroscopic rice bodies, no granulomas	1. ND 2. ZN-, culture synovial fluid - 3. Culture biopsy + <i>M. avium</i>	1. Cystectomy 2. Puncture 3. Debridement	Clarithromycin 7m + ethambutol 1m
F/60	Minor hand trauma	Swelling index and third finger, wrist nodules on forearm (sporotrichoid)	6 months	At onset none, initial diagnosis RA treated with corticosteroids	Fish tank	Ultrasound: soft tissue swelling RX: normal	Chronic synovitis, possible granulomas	ZN -, culture + <i>M. marinum</i>	Debridement	Azithromycin 3m + ethambutol 3m
F/80	Minor leg trauma, small open wounds	Papulous skin lesions and noduli on the leg (sporotrichoid)	5 months	RA receiving corticosteroids, diabetes type 2	Outdoor, gardening, use of outdoor well	None	Granulomas	ZN +, culture + <i>M. chelonae- abscessus</i> complex	None	Clarithromycin 7m + linezolid 1 week, + cotrimoxazole 3 m
M/81	Minor trauma on thumb	Swelling, redness of thumb and hand	4 months	None	Partner pulmonary TBC	Ultrasound: tenosynovitis	Granulomas	ZN -, culture + <i>M. tuberculosis</i> complex	Tenolysis	Rifampicin 6m + isoniazide 6m + pyrazinamide 2m

ND = not determined; NTM = non-tuberculous mycobacteria; RA = rheumatoid arthritis; TBC = Tuberculosis; ZN = Ziehl-Neelsen stain; m = months

diagnosis was only arrived at after surgical debridement revealing macroscopic rice bodies and mycobacterial culture of deep tissue biopsies revealing the presence of *M. avium*. She was treated with clarithromycin 500 mg b.i.d. for seven months, in association with ethambutol 1200 mg q.d. during the first month, which was stopped after four weeks when the strain appeared resistant. The lesions healed, despite persistent swelling of the little finger as long-term sequel.

### Case 2

A 60-year-old woman developed swelling and stiffness of two fingers of the right hand after a minor trauma. Based on histological examination of a tissue biopsy, a working diagnosis of rheumatoid arthritis was made, and treatment was initiated with salazopyrine 500mg b.i.d. The symptoms persisted, however, and she developed painless ascending subcutaneous nodules on the right forearm (figure 1C). One month after surgical debridement,

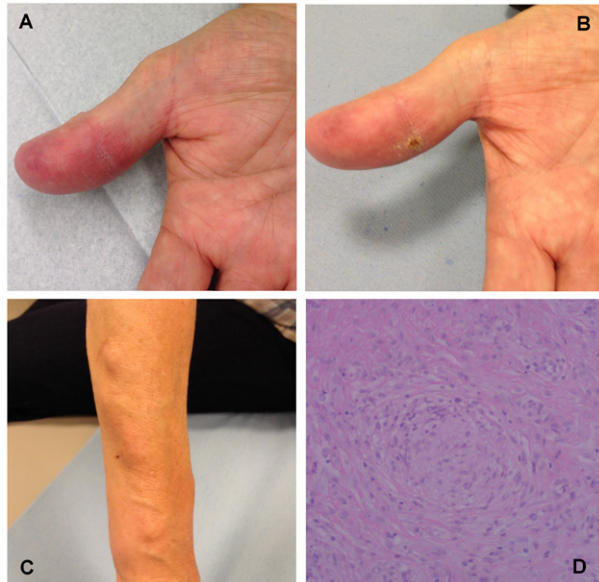
mycobacterial cultures revealed *M. marinum*. Salazopyrine was stopped and she was treated with azithromycin 500 mg q.d. and ethambutol 1200 mg q.d. during four months, resulting in full recovery.

### Case 3

An 80-year-old woman was referred to our clinic five months after she developed swelling and ulcers on the right lower leg and foot after a minor trauma. She was known with polymyalgia rheumatica for which she received corticosteroids (methylprednisolone 8 mg q.d.). Since the lesions persisted for more than 5 months, a skin biopsy and culture was performed which yielded *M. chelonae- abscessus* complex. No further surgical procedures were performed. Antimycobacterial treatment was initiated consisting of clarithromycin 500 mg b.i.d. in combination with linezolid 600 mg b.i.d., and corticosteroids were discontinued. Linezolid was stopped after two weeks due to toxicity while clarithromycin was continued for seven

**Figure 1.** Clinical and anatomopathological pictures of the presented case series

Swelling and redness of the right hand and thumb with tenosynovitis caused by *M. tuberculosis* at presentation (figure 1A) and after 4 months of treatment (figure 1B); sporotrichoid nodules progressing along the lower arm, due to *M. marinum* (figure 1C); synovium biopsy showing epithelioid granuloma, giant cells and epithelioid histiocytes caused by *M. tuberculosis* (figure 1D)



months. However, two months after discontinuation a relapse occurred, triggered by high doses of corticosteroids for a bout of polymyalgia rheumatica. A new culture of a lesion confirmed the mycobacterial relapse. Clarithromycin and linezolid were restarted, but linezolid had again to be discontinued due to gastro-intestinal toxicity. The patient was subsequently hospitalized during three months for IV treatment with imipenem 500 mg q.i.d. associated with tobramycin 5 mg/kg q.d. adjusted by drug monitoring. Corticosteroids were tapered and stopped. Clarithromycin 500 mg b.i.d. and cotrimoxazole 800/160 mg b.i.d. were prescribed for another three months. A long revalidation phase ensued characterized by recurring granulomatous draining inflammatory lesions, but without growth of mycobacteria, considered a paradoxical reaction. Complete recovery was obtained.

**Case 4**

An 81-year-old man presented with swelling and redness of his right hand and thumb for four months after a minor trauma (figure 1A and 1B). He regularly cleaned his fish tank without wearing gloves. He also took care of his wife, who was diagnosed with pulmonary tuberculosis. Tenolysis was performed and tissue biopsy revealed granulomas (figure 1D); cultures yielded *M. tuberculosis* complex. A skin

tuberculin test was positive. A chest X-ray showed a pleural effusion from which *M. tuberculosis* could be cultured. Treatment with rifampicin 300 mg b.i.d. and isoniazide 300 mg q.i.d. during six months, in association with pyrazinamide 2000 mg q.d. during the first two months, was initiated. The patient made full recovery.

**DISCUSSION**

We present four clinically similar cases of mycobacterial skin and soft tissue infection caused by different tuberculous and nontuberculous mycobacterial species, all characterised by long delay in diagnosis and appropriate treatment. These infections are rare, hence not readily recognized. We performed a literature search on mycobacterial tenosynovitis using the Medline terms 'mycobacterium AND tenosynovitis'. The search was limited to case series of at least three patients, published on or after 1 January 1997, written in English and clinically sufficiently detailed. Referenced articles were searched for additional case series. Results are summarized in table 2.<sup>5-21</sup> Of the 162 hits, only 20 fulfilled the criterion of at least three patients, three of those were excluded for lack of sufficient clinical information.

In four of the 17 remaining case series tenosynovitis was caused by *M. tuberculosis*, in 12 by non-tuberculous mycobacteria (table 2). One case series concerned *M. bovis* tenosynovitis, a mycobacterium belonging to the *M. tuberculosis* complex species best known as the causative agent of tuberculosis in cattle. Overall mycobacterial tenosynovitis appears to occur in a wide range of the population with a mean age at time of infection ranging from 32-72 years, also reflecting different exposure risks such as occupation. The use of immunosuppressive medication is a risk factor for both tuberculous and non-tuberculous mycobacterial infection,<sup>22</sup> but most patients are immunocompetent, as illustrated by our cases and the case series selected, only three of which involve immunocompromised patients.<sup>7,12,13</sup>

As non-tuberculous mycobacteria are ubiquitous environmental species, skin and soft tissue infections usually result from direct inoculation of the microorganism, as is illustrated in a recent outbreak of *M. chelonae* cutaneous infections resulting from contaminated ink used for tattoos.<sup>23</sup> Similarly, *M. marinum* is a pathogen associated with fishes and aquatic exposure.<sup>24</sup> Furthermore, as illustrated in our cases and corroborated by the selected case series concerning non-tuberculous mycobacterial tenosynovitis, a preceding minor trauma has been frequently identified as an exposure risk. In contrast, *M. tuberculosis* infections generally result from person-to-person transmission. Therefore in taking medical history it should be explored whether patients with suspected

**Table 2.** Overview of published case series of mycobacterial tenosynovitis as from 1997 (minimum 3 patients)

Ref	Country	Number of tenosynovitis cases	Years of diagnosis	Mean age	Exposure to NTM/TBC	Mean diagnostic delay	Ziehl-Neelsen stain	Culture	PCR
Yao 2017	USA	3	2013	72	3/3 aquatic exposure, 1/3 trauma	4 months	ND	All negative	3/3 <i>M. marinum</i>
Kabakas 2015	Turkey	13	2001-2010	32	1 partner pulmonary TBC	5 months	13/13 negative	9/13 <i>M. tuberculosis</i>	ND
Johnson 2015	USA	18	1996-2014	55	13/18 aquatic exposure, 4/18 immunocompromised	4 months	2/18 positive	18/18 <i>M. marinum</i>	ND
Guner 2014	Turkey	3	ND	60	3/3 contact with cattle	ND	ND	3/3 <i>M. bovis</i>	ND
Hsiao 2013	Taiwan	17	2001-2010	52	9/17 trauma, 7/17 aquatic exposure	6 months	3/11 positive	7/17 <i>M. marinum</i> , 5/17 <i>M. intracellulare</i> , 2/17 <i>M. abscessus</i> , 1/17 <i>M. haemophilum</i> , 1/17 <i>M. arupense</i> , 1/17 <i>M. terrae</i>	ND
Yano 2013	Japan	5	1999-2006	57	2/5 trauma	33 months	5/5 negative	3/5 <i>M. marinum</i> , 1/5 <i>M. intracellulare</i> , 1/5 <i>M. kansasii</i>	ND
Cheung 2012	China	166	1981-2009	50	166/166 trauma, 110/166 aquatic exposure	5 months	ND	67/166 <i>M. marinum</i>	ND
Woon 2011	Singapore	6	1998-2006	54	2/6 immunocompromised	4 months	3/4 positive	4/6 <i>M. tuberculosis</i>	1/1 <i>M. tuberculosis</i>
Bauer 2010	USA	3	2004-2009	69	3/3 TNFa inhibitors, 3/3 trauma	ND	3/3 negative	1/3 <i>M. mucogenicum</i> , 1/3 <i>M. marinum</i>	ND
Kotwal 2009	India	7	2003-2007	24	ND	ND	ND	<i>M. tuberculosis</i> , number not mentioned	performed in equivocal cases
Pang 2007	Singapore	5	2001-2006	38	5/5 aquatic exposure, 5/5 trauma	2 months	5/5 negative	5/5 <i>M. marinum</i>	ND
Rashid 2006	Pakistan	3	2004-2005	43	ND	ND	ND	2/3 <i>M. tuberculosis</i>	ND
Hassanpour 2006	Iran	12	1991-2001	38	ND	ND	8/10 positive	10/10 <i>M. tuberculosis</i>	ND
Tsai 2006	Taiwan	3	2004-2005	57	3/3 trauma	7 months	3/3 positive	3/3 <i>M. marinum</i>	2/2 <i>M. marinum</i>
Noguchi 2005	Japan	5	ND	67	2/5 aquatic exposure, 3/5 farmers	7 months	ND	3/5 <i>M. marinum</i> , 2/5 <i>M. intracellulare</i>	ND
Pratt 2005	Australia	3	2004	ND	3/3 trauma	ND	ND	2/3 <i>M. marinum</i> , 1/3 <i>M. kansasii</i>	ND
Chau 2003	China	3	2000-2002	65	ND	13 months	3/3 negative	3/3 <i>M. avium-intracellulare complex</i>	ND

mycobacterial skin infections have recently been in contact with patients suffering from or treated for tuberculosis. After infection, hematogenous spread to a musculoskeletal location is considered to be the most common pathway.<sup>2,3</sup> However, in our case 4, involving lung tuberculosis of the patient's wife, inoculation in the thumb due to local trauma should be considered the porte d'entrée, similar to non-tuberculous tenosynovitis.

Spreading of lesions is typically slow and progresses over a period of weeks to months. In many patients there is a typical sporotrichoid spread, i.e. the appearance of subcutaneous nodules that progress along dermal and lymphatic vessels (*figure 1C*). As cutaneous sporotrichosis, a rare fungal infection which has a similar clinical presentation, does not occur in Europe, mycobacterial infection should be suspected.<sup>25</sup> In all patients, however, there is a considerable delay in diagnosis ranging from five up to 12 months. In each patient of our case series, there were clinical clues that could have led to an earlier diagnosis, such as specific underlying predilection factors, typical clinical features and histology.

Diagnosing mycobacterial tenosynovitis is a difficult and often lengthy process. Biochemical analysis does not contribute to the diagnosis, but imaging studies can be of use: ultrasound or MRI may show the presence of 'rice bodies', as exemplified by the case series published by Woon et al.<sup>12</sup> and Chau et al.<sup>21</sup> Nevertheless this was not the case with our four patients. A tuberculin skin test has a low sensitivity and is insufficiently able to distinguish between *M. tuberculosis* and non-tuberculous mycobacteria. As a consequence the positive result of this test did not contribute to the diagnosis in our cases, nor did it appear to be useful in the selected case series. An interferon-gamma release assay is better at detecting *M. tuberculosis* although there still is cross-reactivity with a few non-tuberculous mycobacteria, such as the frequently encountered *M. marinum*.<sup>26</sup>

For a definite diagnosis of mycobacterial skin and soft tissue infection an appropriate tissue biopsy is required. This can show a typical granulomatous inflammation (*figure 1D*). In our patient 1 surgical debridement revealed macroscopic rice bodies, which can be another clue to the diagnosis. Rice bodies are a nonspecific response to inflammation, macroscopically resembling polished rice. They are commonly found in synovial lavage of rheumatoid joints.<sup>12</sup> However, when found in tenosynovium, a mycobacterial cause should be suspected.<sup>27</sup> The most important contribution comes from cultures of deep tissue biopsies, as the anatomopathological finding of granulomas, and especially Ziehl-Neelsen staining and synovial fluid cultures have low sensitivity.<sup>2</sup> In case of suspicion of mycobacterial infection this should be mentioned when providing tissue to the laboratory, since culturing these bacteria requires adapted processing,

especially in the case of non-tuberculous mycobacteria, where less severe decontamination procedures and lower incubation temperatures are necessary.<sup>4,28</sup>

Because antibiotic susceptibility of non-tuberculous mycobacteria is unpredictable and variable, and *M. tuberculosis* requires specific combinations of antibiotics, the optimal treatment can only be initiated once a definite microbial examination has been performed, which usually takes several weeks. Molecular diagnostic approaches as polymerase chain reaction (PCR) for *M. tuberculosis* can lead to a faster diagnosis, but the value of this approach for non-tuberculous mycobacteria is less clear, partly because of their ubiquitous presence in the environment, leading to contamination.<sup>2</sup> Interestingly, this did not lead to a shorter diagnostic delay in the four case series where PCR was performed, most likely because this technique was only used after cultures remained negative. The treatment of non-tuberculous infections is not analogous to the treatment of *M. tuberculosis* infections and choosing the correct treatment is difficult, partly because of the bad correlation between *in vitro* susceptibility and clinical response for many non-tuberculous mycobacteria. ATS/IDSA guidelines provide excellent guidance on treatment of these infections, bearing in mind that the recommendations for infrequently encountered mycobacteria are based on few reported cases, emphasizing the importance of case reports and case series.<sup>4,29</sup>

## SUMMARY

Both nontuberculous and tuberculous mycobacteria can cause chronic tenosynovitis. An investigation into predilection factors and exposure risks is mandatory. A correct species determination based on deep tissue biopsy cultures is important for guiding subsequent treatment. We present four cases with similar clinical presentation, of which three were caused by nontuberculous and one by tuberculous mycobacteria, and review the relevant literature.

## CONCLUSION

In conclusion, even in high resource countries, *M. tuberculosis* must be considered a causative agent of chronic tenosynovitis next to the more common non-tuberculous mycobacterial skin and soft tissue infections. In all mycobacterial infections, actively searching for predilection factors, exposure risks and clinical clues is clearly indicated. Deep tissue biopsy cultures are indispensable to identify granulomas and to determine the species and strain of mycobacterium, which directs the duration and choice of antimycobacterial treatment.



## DISCLOSURES

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# The treatment outcomes in IgG4-related disease

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## ABSTRACT

**Introduction:** IgG4-related disease (IgG4-RD) is an emerging systemic inflammatory disease involving nearly all organs eventually leading to fibrosis. Prompt and adequate treatment to prevent irreversible organ damage is therefore pivotal. To evaluate the treatment outcomes, we studied a well-defined cohort of patients with IgG4-RD. **Method:** 32 patients with histologically confirmed IgG4-RD diagnosed between 1999 and April 2017 were included and reviewed for demographic and clinical characteristics. The response to treatment with glucocorticoids, disease modifying antirheumatic drugs, rituximab and other therapeutic interventions were evaluated.

**Results:** Glucocorticoids as well as rituximab appeared successful therapeutic drugs leading to clinical remission (complete or partial remission) in all patients. Recurrences, however, were frequently seen (62% versus 100%, respectively). Diseases modifying antirheumatic drugs (DMARDs), including azathioprine, methotrexate and mycophenolate mofetil were effective in less than half of the cases. A minority of patients was treated with alternative treatments including hydroxychloroquine, thalidomide and infliximab which all appeared effective. Surgical intervention and radiotherapy in local disease seemed to induce clinical remission and were associated with low recurrence rates.

**Conclusion:** Glucocorticoids and rituximab induce substantial responses as well as primary surgical intervention and radiotherapy, while the efficacy of DMARDs is limited. Based on the few data available, hydroxychloroquine, infliximab and thalidomide may be promising treatment options for second or third line strategies.

## KEYWORDS

IgG4-related disease, DMARDs, azathioprine, hydroxychloroquine, mycophenolate mofetil, rituximab, methotrexate, infliximab, clinical outcomes

## INTRODUCTION

IgG4-related disease (IgG4-RD) is a systemic fibro-inflammatory disease potentially affecting all parts of the human body.<sup>1</sup> Eventually fibrosis may lead to irreversible organ damage and even secondary amyloidosis may occur.<sup>2</sup> Therefore early recognition and swift initiation of adequate therapy remain critical.<sup>3,5</sup>

The pathogenesis of IgG4-RD is still unclear and the trigger causing the inflammation seen in IgG4-RD is unknown, but recently evolving knowledge is leading to a better understanding of the disease. The elevated IgG4 levels and the good response of IgG4-RD patients to treatment with rituximab (B-cell depletion treatment), suggest a role of the humoral immune system in the pathogenesis of IgG4-RD.<sup>6,7</sup> IgG4 positive B-cells have been studied in IgG4-RD demonstrating increased numbers of blood IgG4 positive B-cells in patients compared to controls.<sup>8,9</sup> In addition, oligoclonally circulating total plasmablasts are increased and appear to play a role in IgG4-RD, while the number of plasmablasts decreased after B-cell depletion.<sup>6,10,11</sup> These plasmablasts show extensive somatic hypermutation (SHM) in the rearranged variable regions, which indicates a T-cell dependent response.<sup>11,12</sup> Different T-cell subsets have been studied in IgG4-RD and have shown that T-cells are also important in the pathogenesis of IgG4-RD.<sup>6,9,13</sup> T follicular helper 2 (Tfh2) cells are possibly involved in driving the class switch to IgG4.<sup>14</sup> Different cytokines,

including interleukin (IL)-4 derived from T-helper 2 (Th2) cells may also contribute to the pathophysiology of IgG4-RD.<sup>9,15-17</sup> but the role of Th2 cells in IgG4-RD remains unclear.<sup>18,19</sup> Recently, CD4+ T-cells displaying cytotoxic features appeared to be abundantly present in peripheral blood and diseased tissue sites of patients with IgG4-RD and possibly also contribute to the pathogenesis of the disease.<sup>13,20</sup>

Glucocorticoids are the first choice for treatment. The relapse rate after tapering glucocorticoids is high, hence steroid-sparing maintenance therapy is often required.<sup>21</sup> Several disease modifying anti-rheumatic drugs (DMARDs) such as methotrexate and azathioprine have been used as steroid-sparing treatment of IgG4-RD, but studies confirming their efficacy are lacking.<sup>22</sup> Most of the reports on the use of steroid-sparing treatment with DMARDs are case-based reports.<sup>23</sup> Emerging data from case series reveal promising results for rituximab in the treatment of patients with IgG4-RD.<sup>1,24-26</sup> The aim of the current study is to evaluate the different treatment outcomes in a well-defined cohort of patients with IgG4-RD.

## MATERIALS AND METHODS

The Erasmus University Medical Center is a tertiary referral centre for patients with IgG4-RD. All patients diagnosed with IgG4-RD are treated and monitored prospectively. However, patients may also be diagnosed with IgG4-RD retrospectively. Medical records of patients with IgG4-RD between 1999 and April 2017 were reviewed for demographic and clinical characteristics. Only patients with histologically confirmed IgG4-RD according to established Boston criteria for histology were included.<sup>27</sup> The efficacy of all therapies including glucocorticoids, DMARDs (mycophenolate mofetil, methotrexate and azathioprine), hydroxychloroquine, cyclophosphamide, rituximab, thalidomide, infliximab, surgery and radiotherapy were evaluated. The disease activity in patients was evaluated using the IgG4-related disease Responder Index (IgG4-RD RI), a monitoring tool for disease activity in IgG4-RD using the clinical, laboratory and radiological outcomes. IgG4-RD RI is designed for the physicians to easily score the extent of the disease activity. A score of 0 signifies the absence of active disease in an organ, a score of 1 indicates improvement of the disease activity within an organ, a score of 2 indicates that the disease within an organ has remained unchanged, a score of 3 indicated the presence of new or recurrent disease activity and a score of 4 refers to disease that has worsened despite treatment. The levels of serum IgG4 are scored as same manner and scored according to normal,

improved, persistent or new/recurrent/worsened despite treatment.<sup>28</sup>

Active disease was defined by an IgG4-RD RI score of  $\geq 3$ .<sup>29,30</sup> Improvement in the disease activity and complete response are defined as a decline of  $\geq 2$  points compared to the baseline score or IgG4-RD RI of  $< 3$  and decline  $\geq 2$  after treatment, respectively. Partial response and disease relapse were defined as a decline of  $\geq 2$  points in IgG4-RD RI, but still  $\geq 3$  and worsening of clinical, radiological and serological (serum IgG4) findings, respectively.

This study was performed according to the Declaration of Helsinki and was approved by the Medical Ethics Committee of Erasmus MC (ethics approval numbers MEC-2017-1169).

## RESULTS

All together 32 patients with IgG4-related disease were included with a mean age of 57 years, ranging from 17 to 77 years, of which 72% were male. The main outcomes of this study are presented in *table 1*. An overview of the different treatment modalities in these 32 patients is presented in *table 2A* and the different treatment outcomes (response, failure and relapse) for each treatment strategy are demonstrated in *table 2B*.

The patients represented a heterogeneous group of IgG4-RD with different organ manifestations. Most of the patients had ocular (53%) manifestation of the disease. The medical history was unremarkable in most of the cases, though five patients had asthma. All the cases were histologically confirmed using the Boston consensus criteria for histology.<sup>27</sup>

Almost all patients (29/32 = 91%) were treated with glucocorticoids (mostly prednisone 0.5-1 mg/kg/day) leading to clinical response in all. In the remaining three patients, initial treatment with methotrexate followed by hydroxychloroquine in one patient and surgery in two patients led to complete response. In 27 (out of 29) patients glucocorticoids were started initially and in two after relapse or failure of the initial glucocorticoid sparing regime (patients 4 and 6). In 10 (out of 27) patients glucocorticoids were initiated as monotherapy; in the remaining patients these were combined (*table 2A*).

Complete response was observed in 72% of the patients receiving glucocorticoids, whereas 28% showed a partial response. Glucocorticoids were usually continued for a period of four to six weeks and thereafter tapered slowly and withdrawn in a period of three months up to one year. In just two patients a short course of prednisone was started after initial surgical excision of the affected tissue. Despite a good initial clinical response a flare of the disease occurred when the glucocorticoids were tapered

**Table 1.** Patient characteristics and treatment outcomes, treatment failures, side effects and current treatment of patients

Patient characteristics											
Patient	Age	Sex	Medical history	Diagnosis	Elevated serum IgG4	Initial treatment (IgG4-RD RI at presentation)*	Therapy failure	Therapy side effects	Therapy response	Relapse after therapy	Current status / treatment
I	60y	F	-	IgG4-related skin disease	Yes	Methotrexate (4)	Methotrexate	Methotrexate (liver toxicity)	Hydroxy-chloroquine (CR)	NA	Hydroxy-chloroquine
2	55y	M	Asthma	IgG4-related orbital, lymph node and skin disease	Yes	Prednisone (8)	-	-	Prednisone (PR)	Prednisone	Prednisone. Rituximab is being considered
3	38y	M	-	IgG4-related salivary disease	Yes	Prednisone and azathioprine (4)	Mycophenolate mofetil, azathioprine	Thalidomide (neuropathy)	Prednisone (CR), rituximab (PR), thalidomide (CR)	Prednisone, rituximab	Thalidomide on demand due to neurological side effects
4	45y	M	-	IgG4-related orbital disease	Yes	Hydroxy-chloroquine (4)	Hydroxychloroquine	-	Prednisone (CR)	NA	No treatment
5	65y	M	-	IgG4-related orbital disease and scleritis	No	Prednisone and azathioprine (4)	-	Azathioprine (liver toxicity)	Prednisone (CR)	NA	No treatment
6	43y	M	2006: cervical follicular B-cell lymphoma: later revised to IgG4-RD	IgG4-related lymph node and renal disease	Yes	Radiotherapy (3)	-	-	Radiotherapy (CR), prednisone (PR), rituximab (CR), mycophenolate mofetil (PR)	Radiotherapy, prednisone, rituximab	Mycophenolate mofetil and low dose prednisone
7	68y	F	Carpal tunnel syndrome	IgG4-related orbital disease	Yes	Prednisone and Mycophenolate mofetil (6)	Mycophenolate mofetil	-	Prednisone (CR), rituximab (CR)	Prednisone tapering, rituximab	Dexamethasone tapering
8	77y	M	Asthma, hypertension	IgG4-related skin and lymph node disease	Yes	Prednisone and azathioprine (8)	-	Azathioprine (liver toxicity)	Prednisone (CR), rituximab (CR)	Prednisone, rituximab	Rituximab every 6 months
9	61y	M	-	IgG4-related orbital and lymph-node disease	Yes	Prednisone and methotrexate (6)	Methotrexate	-	Prednisone (CR)	Prednisone	Rituximab is being initiated
10	63y	F	-	IgG4-related orbital disease with epilepsy, vision loss and trismus	Yes	Prednisone (4)	Methotrexate, cyclosporine A, cyclophosphamide	Prednisone (diabetes mellitus)	Prednisone (CR), infliximab (CR)	Prednisone	Infliximab
11	64y	M	Diabetes mellitus type 2 and asthma.	IgG4-related orbital, lymph-node, pancreas and prostate disease	Yes	Prednisone and methotrexate (10)	-	-	Prednisone (PR), methotrexate (PR)	NA	Methotrexate

Patient characteristics											
Patient	Age	Sex	Medical history	Diagnosis	Elevated serum IgG4	Initial treatment (IgG4-RD RI at presentation)*	Therapy failure	Therapy side effects	Therapy response	Relapse after therapy	Current status / treatment
12	60y	M	Diabetes mellitus type 2 and asthma	IgG4-related orbital, lymph node, pancreas and prostate disease	Yes	Prednisone and azathioprine (10)	-	Azathioprine (gastro-intestinal toxicity)	Prednisone (CR)	Prednisone	Prednisone. Rituximab is being considered
13	64y	M	Hypothyroidism	IgG4-related orbital, lymph node, thyroid, prostate and pancreas disease	Yes	Prednisone (10)	-	-	Prednisone (CR)	Prednisone	Prednisone. Rituximab is being considered
14	17y	M	-	IgG4-related lung, lymph node and cerebral disease	Yes	Dexamethasone and azathioprine (8)	Azathioprine	-	Dexamethasone (PR), rituximab (PR)	Dexamethasone, rituximab	Rituximab every 6 months
15	63y	F	Hypothyroidism and COPD Gold 1	IgG4-related pancreatitis, lung, lymph node and salivary gland disease	No	Partial pancreas resection followed by prednisone (8)	-	-	Surgery (CR), prednisone short course (CR)	NA	No treatment
16	54y	M	Mesenteric mass for 16 years	IgG4-related mesenteric disease	Yes	Prednisone and azathioprine (4)	Azathioprine	-	Prednisone (PR), rituximab (PR)	Prednisone, rituximab	Methyl-prednisolone monthly
17	63y	M	Optic neuritis	IgG4-related thyroid disease	Yes	Hemithyroidectomy followed by prednisone (6)	-	-	Surgery (CR), prednisone short course (CR)	NA	No treatment
18	59y	M	-	IgG4-related lung disease and ocular disease	No	Surgical resection lung lesion followed by prednisone and methotrexate (4)	-	-	Surgery (PR), prednisone (CR), methotrexate (CR)	NA	No treatment anymore
19	33y	M	-	IgG4-related pericarditis and pleural disease	Yes	Pericardiectomy followed by prednisone (8)	-	-	Surgery (PR), prednisone (CR)	NA	No treatment
20	42y	M	-	IgG4-related orbital and salivary disease	Yes	Prednisone (6)	Azathioprine	-	Prednisone (CR), hydroxychloroquine (CR)	Prednisone tapering	Hydroxychloroquine
21	42y	F	-	IgG4-related periorbital disease and scleritis	No	Prednisone and azathioprine (4)	-	Azathioprine (muscle pain)	Prednisone (CR)	NA	No treatment

Patient characteristics											
Patient	Age	Sex	Medical history	Diagnosis	Elevated serum IgG4	Initial treatment (IgG4-RD RI at presentation)*	Therapy failure	Therapy side effects	Therapy response	Relapse after therapy	Current status / treatment
22	70y	M	Myocardial infarction	IgG4-related orbital, nose and salivary gland disease	Yes	Prednisone (8)	-	-	Prednisone (CR)	NA	No treatment
23	60y	M	COPD Gold I	IgG4-related cholangitis, lymph node and nasal disease	Yes	Prednisone (8)	Methotrexate	Azathioprine (gastro-intestinal toxicity) Hydroxy-chloroquine (gastro-intestinal toxicity)	Prednisone (CR)	Prednisone	Prednisone. Rituximab being considered
24	65y	M	-	IgG4-related orbital, lymph node and pancreas disease	Yes	Prednisone (8)	-	-	Prednisone (PR)	Prednisone	Prednisone. Additional treatment is being considered
25	74y	F	Asthma, acute rheumatic fever	IgG4-related lung diseases	Yes	Surgical resection lung lesion (4)	-	-	Surgery (CR)	NA	No treatment after surgery
26	65y	M	Benign prostate hypertrophy	IgG4-related retroperitoneal fibrosis	Yes	Prednisone (6)	-	Azathioprine (gastro-intestinal toxicity)	Prednisone (CR)	Prednisone	No treatment
27	53y	M	Chronic spontaneous urticaria	IgG4-related orbital and nasal disease	Yes	Prednisone and methotrexate (6)	Methotrexate	-	Prednisone (CR)	Prednisone	Rituximab is being initiated
28	73y	M	Prostate carcinoma for which radiotherapy	IgG4-related orbital and nasal disease	No	Prednisone and azathioprine (6)	Azathioprine, cyclophosphamide	-	Prednisone (PR), surgery (enucleation = CR)	Prednisone tapering	No treatment after surgery
29	50y	F	-	IgG4-related orbital disease	No	Prednisone (4)	-	-	Prednisone (CR)	NA	No treatment
30	54y	F	-	IgG4-related orbital disease	No	Prednisone (2)	-	-	Prednisone (PR), cyclophosphamide (CR) Radiotherapy (CR)	Prednisone, cyclophosphamide	No treatment after radiotherapy
31	71y	M	-	IgG4-related pancreatitis and lymph node disease	Yes	Prednisone and azathioprine (8)	-	-	Prednisone (CR), azathioprine (CR)	NA	Azathioprine
32	61y	F	-	IgG4-related lung disease presenting as recurrent tumors	No	Surgical resection lung lesions (6)	-	-	Surgery (CR)	NA	No treatment after surgery

CR = complete response; PR = partial response; IgG4-RD RI = IgG4-Related Disease Responder Index

\*IgG4-RD RI at baseline. The activity scores during the follow-up are not shown, but are available upon request

**Table 2.** An overview of the different treatment modalities

**Table 2A.** The different treatment modalities in patients with IgG4-related disease

Initial therapy	N = 32	Additional therapy	Maintenance therapy
Glucocorticoids	10	Glucocorticoids (n = 7) Glucocorticoids + cyclophosphamide + radiotherapy (n = 1)	6 (60%): Glucocorticoids (n = 4) Hydroxychloroquine (n = 1) Infliximab (n = 1)
Glucocorticoids + Methotrexate	3	Glucocorticoids (n = 2)	3 (100%): Glucocorticoids (n = 2) Methotrexate (n = 1)
Glucocorticoids + Azathioprine	9	Glucocorticoids (n = 1) Glucocorticoids + surgery (n = 1) Glucocorticoids + rituximab (n = 4)	5 (67%): Azathioprine (n = 1) Glucocorticoids (n = 2) Rituximab (n = 1) Thalidomide on demand (n = 1)
Glucocorticoids + Mycophenolate mofetil	1	Glucocorticoids + rituximab	1 (100%): Glucocorticoids
Methotrexate	1	Hydroxychloroquine	1 (100%): Hydroxychloroquine
Hydroxychloroquine	1	Glucocorticoids	0
Surgery	2	0	0
Surgery + glucocorticoids	3	0	0
Surgery + glucocorticoids + methotrexate	1	0	0
Radiotherapy	1	Glucocorticoids, rituximab, mycophenolate mofetil	1 (100%): Mycophenolate mofetil

**Table 2B.** Response to different treatment modalities in patients with IgG4-related disease

Therapy	N = 32, including 76 treatment episodes	Complete response	Partial response	Therapy failure	Relapse after initial response	Side effects
Glucocorticoids	29	21 (72%)	8 (28%)	0	18 (62%)	1 (3%)
Methotrexate	7	1 (14%)	1 (14%)	5 (71%)	0	1 (14%)**
Azathioprine	12	1 (8%)	0	5 (42%)	0	6 (50%)
Mycophenolate mofetil	3	0	1 (33%)*	2 (67%)	0	0
Hydroxychloroquine	4	2 (50%)	0	1 (25%)	0	1 (25%)
Cyclophosphamide	3	1 (33%)	0	2 (67%)	1 (33%)	0
Thalidomide	1	1 (100%)	0	0	NA	1 (100%)
Cyclosporine A	1	0	0	1 (100%)	NA	NA
Infliximab	1	1 (100%)	0	0	0	0
Rituximab	6	3 (50%)	3 (50%)	0	6 (100%)	0
Surgery	7	5 (71%)	2 (29%)	0	0	0
Radiotherapy	2	2 (100%)	0	0	1 (50%)	0

NA = not applicable (because treatment was discontinued)\*In combination with low dose prednisone  
\*\*A patient with therapy failure and gastro-intestinal side effects

or discontinued in 62% of the patients, necessitating additional (steroid-sparing) treatment.

Methotrexate was initiated in seven patients, usually in a dose of 15 mg per week, which proved effective in a minority of two (29%, one complete response and one partial response). In the five other patients methotrexate failed to suppress the disease activity, in one case also causing gastro-intestinal toxicity.

A total of 12 patients were treated with azathioprine, with only one complete response (8%), a patient with pancreas and lymph node involvement in which azathioprine was successfully continued as maintenance treatment after prednisone induction therapy. In six out of 12 patients (50%) azathioprine was terminated because of toxicity (liver, gastro-intestinal, muscle symptoms) before disease activity could be assessed. In the remaining five patients azathioprine proved insufficient to suppress the disease activity and led to discontinuation of this treatment (treatment duration > 3 months).

Mycophenolate mofetil was initiated as maintenance treatment after steroid induction in three patients. Two patients showed treatment failure. One patient, with renal and lymph-node involvement of IgG4-RD refractory to prednisone tapering, azathioprine and rituximab showed a partial response with low dose prednisone and mycophenolate mofetil.

In four patients hydroxychloroquine was used as a maintenance therapy, showing complete response in two (50%), after initial therapy with methotrexate and prednisone respectively. In one (25%) patient initial therapy with hydroxychloroquine was ineffective and replaced by prednisone resulting in clinical remission. In another patient hydroxychloroquine was withdrawn because of gastro-intestinal side effects.

Cyclophosphamide was used in 3 patients with organ function threatening disease after induction treatment with prednisone, which caused complete remission of the disease in one patient (33%), albeit only temporary.

As regards other agents, cyclosporine A was used once without clinical effect. Thalidomide was initiated in one case of therapy refractory salivary disease leading to complete remission of the disease. However, the patient experienced neurological side effects and is using thalidomide now on demand.

Infliximab was used once in a patient with orbital disease refractory to prednisone tapering, methotrexate, cyclosporine A and cyclophosphamide. Infliximab has induced a complete remission and the disease has been in remission for more than five years.<sup>31</sup>

Rituximab was initiated, usually as a third line treatment, in six patients by way of maintenance therapy (50% complete response, 50% partial response). However, the clinical remission after a single dose of 2 grams observed in these patients was temporary. All patients relapsed

within six months to two years after initiating rituximab. Two patients who relapsed after treatment with rituximab are currently receiving rituximab every six months with complete remission of the disease.

Primary surgical intervention in seven patients, including hemithyroidectomy, resection of pulmonary masses, pericardiectomy and partial pancreas resection, led to durable complete responses (median = 36 months). In two cases with solitary pulmonary involvement, resection of the lung lesions was performed and there was no further indication for systemic immunosuppressive therapy. In one case with orbital and nasal involvement, enucleation of the eye was eventually performed because of persistent symptoms of pain after treatment failure with regard to prednisone tapering, azathioprine and cyclophosphamide. The enucleation led to clinical remission and pain symptoms resolved. Diagnosis of IgG4-RD was established retrospectively after enucleation.<sup>32</sup> In two other patients a short course of prednisone after surgery was sufficient to achieve complete remission of the disease. Furthermore, radiotherapy was used in two patients for lymph node involvement and therapy refractory IgG4-related orbital disease respectively, causing complete remission in both cases. The patient with lymphadenopathy developed a recurrence of the lymphadenopathy with new onset renal involvement of the disease a couple of years later, for which the patient started on systemic immunosuppressive therapy. The other patient is in clinical remission five years after radiotherapy.

## DISCUSSION

In this study we describe the treatment outcomes in 32 IgG4-RD patients with various organ manifestations. The observations clearly indicate that the treatment of IgG4-RD can be challenging and must be patient specific. The current study represents a heterogeneous cohort of patients with various organ manifestations. As in most studies, IgG4-RD patients were middle-aged and mostly men.<sup>21</sup> However, in a previously published systemic review we demonstrate that IgG4-RD can also affect children of all ages.<sup>33</sup>

### Glucocorticoids

Glucocorticoids are commonly initiated as immunosuppressive induction therapy. Various types of cells of the immune system, including B and T-cells and macrophages, can be affected by glucocorticoids.<sup>34</sup> Glucocorticoids are considered the mainstay treatment of IgG4-RD and usually effective in a dose of 0.5-1.0 mg/kg/day, depending on severity or organ threatening character of the disease despite the absence of randomized clinical trials.<sup>1,22-35</sup> In the current study a high response rate is followed



by swift recurrences after tapering prednisone at levels corresponding to previous reports.<sup>22</sup> The high response rates warrant the use of glucocorticoids as first line treatment despite the frequent recurrences. Such high recurrence rates emphasize the need of alternative steroid-sparing therapy, which has been advocated in many case series.<sup>22</sup> The results in the current study of alternative treatments are discussed below.

### Azathioprine

Azathioprine is frequently used in immune mediated diseases.<sup>36</sup> Among DMARDs, the use of azathioprine has been most frequently reported in patients with IgG4-RD, especially in IgG4-related pancreatitis.<sup>29</sup> According to the consensus guidance, azathioprine, methotrexate or mycophenolate mofetil may be initiated as prednisone sparing therapy in IgG4-RD.<sup>22</sup> A recent study of 18 patients showed therapeutic efficacy for azathioprine in preventing relapse with IgG4-related pancreatitis.<sup>37</sup> Furthermore, case reports have suggested azathioprine to be effective in several manifestations such as IgG4-related cholangitis, IgG4-related kidney disease and hypophysitis.<sup>38-40</sup> In the presented study however, azathioprine appeared effective in only a minority of cases (8%). The only patient with a clinical response to azathioprine had inflammatory pancreatic disease and enlarged lymph nodes. The remaining 11 patients had disease manifestations without pancreatic involvement. Therefore azathioprine may serve as second line therapy for the subgroup of patients with pancreatic localizations. Remarkably, the continuation of azathioprine was restricted because of toxicity in a substantial number of patients. This toxicity is unfamiliar at such high percentages. It remains of interest whether this is a disease specific complication. Therefore the efficacy of azathioprine in IgG4-RD remains unclear.

### Methotrexate

Except for two small studies suggesting methotrexate as a good steroid-sparing drug,<sup>29,41</sup> its application in IgG4-RD has only been described in limited case reports.<sup>42</sup> Methotrexate affects the function of memory T-cells that produce pro-inflammatory and pro-fibrotic cytokines and is therefore a drug of interest in the treatment of IgG4-RD.<sup>43</sup> In the current study however, methotrexate does not seem to be particularly beneficial, despite its low toxicity.

### Mycophenolate mofetil

Mycophenolate mofetil selectively inhibits cytotoxic T-lymphocytes and is being used as an anti-inflammatory agent. Furthermore, mycophenolate mofetil is related with anti-fibrotic effects possibly due to inhibition of the transforming growth factor beta pathway<sup>44</sup> and can therefore be a potentially interesting drug in the treatment of IgG4-RD. Just like the other DMARDs, mycophenolate

mofetil has not been studied in large cohorts, but has been reported in case reports.<sup>45-48</sup> In the current study, mycophenolate mofetil caused partial remission in combination with low-dose prednisone in only one of three patients without toxicity. The use of mycophenolate mofetil therefore may be evaluated in larger cohorts.

### Hydroxychloroquine

Hydroxychloroquine was initially designed as an antimalarial drug, but because of the accompanying antirheumatic effects it has become widely used in rheumatic and autoimmune disease. The exact role of hydroxychloroquine has not been identified, but it is believed that it has anti-inflammatory and possibly also anti-fibrotic effects.<sup>49-51</sup> In the current study hydroxychloroquine was initiated because of its positive outcomes in other inflammatory diseases like sarcoidosis.<sup>52</sup> There are no published reports on hydroxychloroquine in IgG4-RD, but it is often used in the treatment of other immune mediated diseases. In our study, hydroxychloroquine was used in four cases as maintenance therapy and showed complete response in two patients (50%). Gastro-intestinal complaints led to withdrawal of hydroxychloroquine in one patient (25%). Generally, the drug is not associated with severe adverse effects except for retinal toxicity at higher doses.<sup>53</sup> The efficacy of hydroxychloroquine needs to be evaluated in larger cohorts, but in cases of less severe IgG4-RD it may be useful.

### Cyclophosphamide

Cyclophosphamide has been regarded ineffective in IgG4-RD.<sup>54</sup> However, in a recent study cyclophosphamide yielded a lower relapse rate when combined with glucocorticoids relative to monotherapy with glucocorticoids.<sup>39</sup> In the present study three patients with organ threatening disease were treated with cyclophosphamide and glucocorticoids, because of relapse or failure to respond to DMARDs. Patients treated with cyclophosphamide were those diagnosed with IgG4-RD retrospectively. Only one patient achieved a temporarily complete remission. Use of cyclophosphamide in IgG4-RD should therefore be restricted to cases where no other therapy is available in organ threatening disease.

### Rituximab

The B-cell ablative chimeric monoclonal antibody rituximab is an emergent effective treatment strategy for IgG4-RD.<sup>1</sup> however, large randomized controlled studies have not yet been performed. Despite the good clinical response, the relapse rate after treatment with rituximab is high.<sup>26</sup> In the current study, clinical remission occurred in all six patients treated with rituximab (50% complete response versus 50% partial response). Rituximab was initiated after failure with DMARDs or relapse

after prednisone tapering. However, after single dose treatment with rituximab, the disease recurred in all patients six months to two years after initiating the treatment. One of the patients with severe IgG4-related skin disease is currently treated with rituximab 1000 mg every six months. Another patient with systemic disease and recurrence after rituximab is also being treated with rituximab every six months, a treatment strategy already used in other diseases such as granulomatosis with polyangiitis.<sup>55</sup> As suggested previously,<sup>2</sup> rituximab maintenance treatment is a potentially effective strategy in patients with relapses. This needs to be investigated in future studies.

#### Other therapeutic modalities

Cyclosporine A is a calcineurin inhibitor in which a decrease in interleukin-2 production and, therefore, T-cell proliferation is established. Carbajal et al report on a case of IgG4 related disease with cardiac involvement which was refractory on mycophenolate mofetil, azathioprine, methotrexate, or cyclophosphamide, but in the end could be treated with prednisone in combination with cyclosporine.<sup>56</sup> Cyclosporine A was used once in our study without any clinical effect.

Thalidomide has previously been shown to be effective in two patients with IgG4-related skin disease.<sup>57</sup> Because of therapy refractory salivary disease due to IgG4-RD, one patient in our study was successfully treated with thalidomide. Its mechanism of action is not completely understood but suppression of TNF- $\alpha$  production is considered a possibility.<sup>58</sup> However, neurological side effects of thalidomide may lead to discontinuation of this treatment. Infliximab, a chimeric monoclonal antibody against TNF- $\alpha$ , has only been reported in one case report. In our study, one patient was successfully treated with infliximab after treatment failure with methotrexate, cyclosporine A and cyclophosphamide.<sup>59</sup> Fibrosis, a hallmark of IgG4-RD, has been associated with overexpression of TNF- $\alpha$  and might be a target for infliximab and thalidomide.<sup>59</sup>

#### Surgery

IgG4-RD mostly presents as tumor-like infiltrations and can therefore be treated by surgery in certain cases.<sup>21</sup> Surgical treatment of IgG4-RD often leads to regression of the disease.<sup>60-62</sup> Patients primarily treated with surgical intervention in our study proved recurrence free after long-term follow-up. Numbers are small, but they suggest a favorable outcome, so surgical incision/resection of the disease should be considered.

#### Radiotherapy

Radiotherapy has been reported successful in IgG4-RD,<sup>63,64</sup> in the current study causing complete remission in

one patient with therapy refractory orbital disease and another patient with IgG4-related lymphadenopathy. After a couple of years the patient with lymphadenopathy developed a recurrence, but the patient with orbital disease has been in remission for five years now. In this case, the disease was previously refractory to prednisone and cyclophosphamide but eventually responded very well to radiotherapy. Radiotherapy can be considered as a possible treatment strategy for localized and symptomatic therapy in refractory IgG4-RD.

#### CONCLUSION

In this observational study of 32 patients with IgG4-RD, we demonstrated the different treatment outcomes. The rarity of the disease and its many different manifestations at the time of diagnosis make assessing the optimal treatment into a challenge. This study has some limitations. First of all, all the data are observational. The choice of treatment was based on recommendations in the existing literature and the experience of the prescribing immunologist. Furthermore, patients in this cohort presented with IgG4-RD with different organs involved. The treatment outcome could be different, based on the type of organ involved. Unfortunately the cohort described in this study is too small to perform any sub-analyses. Ideally the choice of treatment would be investigated in a randomized controlled trial. This is a challenge on account of the rarity of the disease.

Nevertheless, this study shows that glucocorticoids and rituximab induce substantial responses, as do primary surgical intervention and radiotherapy. The efficacy of DMARDs is limited. Alternative strategies with hydroxychloroquine, thalidomide and infliximab look promising. More data are needed to confirm these observations, so as to arrive eventually at evidence based treatment guidelines, improving the treatment of patients with IgG4-RD disease.

#### RECOMMENDATIONS

The treatment of IgG4-RD is often challenging. Previously small reports have emphasized the efficacy of DMARDs in IgG4-RD, which unfortunately we did not observe. The current consensus guidance however recommends treatment with DMARDs as a second line treatment option in IgG4-RD patients. Larger prospective studies are required to understand the role of DMARDs in IgG4-RD. Based on current knowledge we recommend the following treatment strategy in IgG4-RD:

Use the IgG4-RD RI to establish the disease activity and to monitor the disease activity;

Check whether there is a treatment indication taking into account that IgG4-RD almost always requires treatment because of the likelihood of secondary complications;

Whenever possible, for example in a patient with single organ manifestation of the disease, surgery is preferred because of the favourable disease course with possibly fewer relapses. Consider a short course of glucocorticoids after surgery;

Glucocorticoids (usually prednisone 0.5-1.0 mg/kg/day or equivalent) are preferred as first line therapy for IgG4-RD. The initial dosage of glucocorticoids should be maintained for two to four weeks. Thereafter, glucocorticoids can be tapered slowly. It is recommended to continue using glucocorticoids for three to six months;

In case of relapse after tapering glucocorticoids, consider DMARDs as a second line treatment. In case of severe disease activity, consider rituximab as second line treatment;

In case of liver and pancreas manifestation of the disease, azathioprine should be considered. In case of other organ manifestations, consider methotrexate, mycophenolate mofetil or hydroxychloroquine if the (vital) organs are not threatened. The patients should be monitored frequently in order to keep tabs on the disease activity and avoid possible side effects of the DMARDs;

Consider radiotherapy if organs are threatened by the mass effect of tumor/IgG4-RD;

Rituximab should be started as a third line treatment of IgG4-RD, or earlier in the treatment course when vital organs are affected. Consider rituximab maintenance therapy after induction of 2 grams of rituximab. Evaluate after a couple of years of treatment whether the maintenance therapy can be discontinued;

Further treatment options include cyclophosphamide, to be considered in therapy refractory cases. Rituximab is clearly preferred over cyclophosphamide. Also In patients with therapy failure thalidomide may be considered.

## DISCLOSURES

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# Syndromic sample-to-result PCR testing for respiratory infections in adult patients

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## ABSTRACT

**Background:** Syndromic sample-to-result (SS2R) polymerase chain reaction (PCR) can rapidly identify causative pathogens of respiratory tract infections (RTI). We evaluated diagnostic accuracy and applicability of one of the current SS2R diagnostics, the FilmArray<sup>®</sup> Respiratory Viral Panel.

**Methods:** We performed a prospective study among adults presenting with symptoms of RTI at the Emergency Department of the University Medical Centre Utrecht (the Netherlands) during the 2016-2017 viral respiratory season. Clinical data were collected. We compared SS2R results on nasopharyngeal swabs to conventional real time PCR, calculated turnaround times (TAT) and explored implementation barriers using questionnaires.

**Results:** 62 Patients were included (64.5 yr [interquartile range (IQR) 44.3-75.0]). SS2R sensitivity was 82.9% [95% confidence interval (CI) 67.9-92.9] and specificity was 95.2% [95% CI 76.2-99.9] for detection of all present viruses (n = 60). Kappa agreement (0.73 [95% CI 0.56-0.90]) was good (p = 0.000). Median SS2R TAT was 2:06 hours [IQR 1:45-3:17] compared to 32:00 hours [IQR 26:50-40:42] of conventional PCR (n = 49, p = 0.000). Ease-of-use and fast TAT were unanimously reported as benefits, and low test capacity with a single SS2R system as drawback.

**Conclusion:** SS2R testing for respiratory viruses offers a rapid and reliable diagnostic method which has great potential for more efficient and targeted management in adult patients with RTI.

## KEYWORDS

FilmArray, PCR, rapid test, respiratory virus, validation

## INTRODUCTION

Respiratory viruses predominate as causative pathogens in patients hospitalized with severe acute respiratory illness (SARI), accounting for up to 50% of microbial etiologies.<sup>1,2</sup> Although antibiotics can be safely withheld in proven viral infections, viral SARI is often treated with antibiotics because viral and bacterial lower respiratory tract infections (RTI) cannot reliably be distinguished based on clinical presentation.<sup>1,3</sup> This leads to unnecessary costs and higher risk of antimicrobial resistance.<sup>4,7</sup> Furthermore, studies have shown reduced mortality when oseltamivir was administered as early as possible (e.g. within two days of symptom onset).<sup>8</sup> Rapid syndromic sample-to-result (SS2R) diagnostics based on multiplex polymerase chain reaction (mPCR), generally generating results within two hours, are promising in accelerating virus and bacterial identification<sup>2</sup> and consequently in targeting antibiotic and antiviral therapy.<sup>9</sup> In addition, rapidly ruling out a viral cause of RTI could lead to more efficient use of in-hospital isolation facilities during the viral respiratory season.

Currently, a wide range of molecular rapid tests is available. Promising commercially available SS2R techniques are Alere I<sup>®</sup> Influenza A&B Nucleic Acid Amplification Test (Abbott), Cobas<sup>®</sup> Liat<sup>®</sup> (Roche Diagnostics), eSensor<sup>®</sup> Respiratory Viral Panel (Genmark), FilmArray<sup>®</sup> Respiratory Panel (BioFire Diagnostics), GeneXpert<sup>®</sup> (Cepheid), and the Luminex<sup>®</sup> xTAG Respiratory Viral Panel (Luminex).<sup>2</sup>

This paper describes a prospective clinical study evaluating the accuracy, turnaround time (TAT) and logistical barriers for the implementation of one of these SS2R diagnostics, the FilmArray<sup>®</sup> Respiratory Panel (from now on SS2R), a FDA cleared /CE marked rapid mPCR for a panel of 17 viruses plus three bacteria commonly found in RTI. The choice for this specific test reflects the choice for this SS2R in our hospital. Apart from the analysis of accuracy and implementation issues, the applicability of the SS2R

is evaluated by calculating the potential impact on use of in-hospital isolation facilities and oseltamivir.

## MATERIALS AND METHODS

### Patient inclusion and microbiological testing

During the peak of the 2016-2017 viral respiratory season (3 January – 4 February 2017),<sup>10</sup> nasopharyngeal swabs were taken from patients (age  $\geq 18$ ) with symptoms of a RTI presenting on the Emergency Department (ED) of the University Medical Center Utrecht (UMCU), a 1042 bed tertiary hospital (The Netherlands). Symptoms of RTI were defined as upper or lower respiratory complaints with acute onset. To obtain informed consent from each patient and calculate TATs of the SS2R, patients were only included during lab opening hours (8 am - 5 pm). Two swabs were taken in parallel, one for the regular diagnostic pathway and one for the experimental diagnostic pathway with the SS2R. The first swab was tested for the most common respiratory viruses (table 2) using conventional real-time polymerase chain reaction (RT-PCR). Conventional RT-PCR for respiratory viruses was performed in nasopharyngeal swabs, nasal washes or bronchoalveolar lavage. Nucleic acids were extracted using the total nucleic acid protocol with the MagNA Pure LC nucleic acid isolation system (Roche Diagnostics, Basel, Switzerland). For detection of RNA viruses, cDNA was synthesized using MultiScribe RT and random hexamers (Applied Biosystems, Foster City, CA). Detection of viral pathogens was performed in parallel, using laboratory developed RT-PCR assays specific for the following viruses: respiratory syncytial virus A and B; influenza virus A and B; parainfluenza virus 1-4; rhinoviruses; adenoviruses; human coronaviruses OC43, NL63, and 229E; human metapneumovirus. RT-PCR procedures were performed as described in earlier literature.<sup>11</sup> In brief, samples were assayed in duplicate in a 25- $\mu$ L reaction mixture containing 10  $\mu$ L of cDNA, 12.5  $\mu$ L of TaqMan Universal PCR Master Mix (Applied Biosystems), 300-900 nmol/l of the forward and reverse primers, and 75-200 nmol/l of each probe. To monitor for inhibition, a fixed amount of an internal control virus (murine encephalomyocarditis virus [RNA virus] and porcine herpesvirus [DNA virus]) was added before extraction.<sup>12</sup> The cycle of threshold (Ct) gives an impression of the quantity of the viral load (i.e. a semi quantitative value). The cut-off value for a positive RT-PCR result was a Ct value  $< 45$ .<sup>13</sup> The second nasopharyngeal swab was tested with the SS2R (FilmArray<sup>®</sup> Respiratory Panel version 1.7). The FilmArray<sup>®</sup> contains all needed reagents in a freeze-dried format for extraction, amplification, and detection steps. Respiratory samples are collected in universal transport media. The FilmArray<sup>®</sup> test was performed according to the manufacturer's

instructions. In brief, prior to run 1 ml of hydration solution and 300  $\mu$ L of respiratory sample was added to the reagent pouch. The pouch was then placed on the FilmArray<sup>®</sup> instrument and the test performed using the FilmArray<sup>®</sup> system. After extraction and purification of all nucleic acids from the sample, a nested multiplex PCR is performed followed by an individual singleplex second-stage PCR reactions to detect the products from the first-stage nested PCR. Both the in-house PCR and FilmArray<sup>®</sup> were performed by the virology laboratory and results were approved by a clinical virologist. Results of the SS2R were not used in routine care.

### Data collection and statistical analysis

Standardized collection of clinical and virological data from ED-presentation and, if applicable, from the following hospital admission was performed manually from the electronic patient charts. Results are given as percentages or median with IQR. The accuracy analysis of the SS2R compared to RT-PCR and a comparison of Ct-values with a t-test, were performed with IBM SPSS Statistics<sup>®</sup> (Version 21.0). Accuracy was calculated per detected virus, e.g. influenza virus, respiratory syncytial virus (RSV), coronavirus, rhinovirus and human metapneumovirus (HMPV), and per sample. Test concordance of the SS2R compared to RT-PCR was presented using sensitivity, specificity and positive and negative predictive values for clinical purposes. Since RT-PCR alone might not be considered a 'gold' standard accuracy (or overall percentage agreement) and a Cohen's Kappa statistic were calculated as well. For the accuracy analysis, final results of both diagnostics were used after retesting in case of invalid results. Samples with discrepant results were retested with both diagnostics, no additional sequencing was done. Ct-values of positive samples were measured by RT-PCR. TATs of both diagnostics were calculated for patients of whom the SS2R could be obtained on the day of sampling, in hours from sampling at the ED until the result was reported to the study team. During the clinical study only one SS2R system was available, precluding the possibility of parallel testing. Separately, potential barriers for implementation, advantages and disadvantages of the SS2R were explored by interviewing laboratory technicians working with the SS2R. Answers to the questions were presented descriptively.

## RESULTS

From January 3<sup>rd</sup> till February 4<sup>th</sup>, a (differential) diagnosis of upper or lower RTI was made in 148 adults at the ED, in 104 of whom RT-PCR was performed. Of these, 56 presented during lab opening hours, making a SS2R possible. Additionally, nasopharyngeal swabs for both

**Table 1.** Baseline characteristics of adult patients with suspicion of an RTI presenting at the ED in whom an in-house PCR and rapid SS2R was performed on a nasopharyngeal swab (n = 62)

Characteristics	n = 62
Age (yr) - median (IQR)	64.5 (44.3-75.0)
Male sex - no. (%)	22 (35.5%)
<b>Comorbidities</b>	
Immunocompromised - no. (%)	25 (40.3%)
Astma or COPD - no. (%)	22 (35.5%)
Chronic heart failure - no. (%)	12 (19.4%)
<b>Disease status at presentation</b>	
Time from first symptoms to hospital presentation (days) - median (IQR)	3.0 (2.0-7.0)*
Fever (temperature $\geq 38.0$ °C) - no. (%)	27 (43.5%)
Oxygen suppletion needed ( $\geq 1$ L) - no. (%)	24 (38.7%)
CRP (mg/l) – normal < 10 mg/l - median (IQR)	58 (21-165)
Infiltrate at radiologic imaging - no. (%)	26 (41.9%)
(Differential) diagnosis RTI after ED presentation - no. (%)	56 (90.3%)
<b>Treatment</b>	
Hospital admission - no. (%), of whom:	39 (62.9%)
- In aerogenic isolation - no. (%)	21 (53.8%)
- Intensive or medium care admission directly after presentation - no. (%)	9 (23.1%)
Antibiotics started at presentation - no. (%)	43 (69.4%)
Oseltamivir started at presentation - no. (%)	20 (32.3%)
<b>Clinical outcomes</b>	
Length of hospital stay, if admitted (days) - median (IQR)	6.0 (3.0-8.0)/34†
Death within hospital stay - no. (%)	5 (8.1%)
CRP = C-reactive protein; IQR = interquartile range; n = number; no = number; PCR = polymerase chain reaction; RTI = respiratory tract infection; SS2R = syndromic sample to result; yr = year. *One missing value because in this patient the duration of symptoms was not reported. Value is calculated using complete cases (n = 61). †5/39 patients were admitted at another hospital due to limited capacity for hospitalization, hence the length of hospital stay of these patients was unknown	

RT-PCR and SS2R were taken from six patients after leaving the ED, who had been referred there with respiratory complaints, but with a non-RTI working diagnosis, so that eventually 62 patients were included. Median age was 64.5 years (IQR 44.3-75.0). Twenty-five patients (40%) were immunocompromised at the time of presentation. Thirty-nine (63%) were admitted to the hospital, nine of whom to the Intensive Care Unit (table 1). Results of the RT-PCR and SS2R showed 58% and 60% samples with one virus detected and 8% and 2% with dual or triple viral pathogens respectively. Most frequented detected viruses were influenza A virus, coronavirus, rhinovirus and RSV (table 2). Viral-bacterial coinfection was present in 14 patients (*Streptococcus pneumoniae* (n = 4), *Haemophilus influenzae* (n = 3), *Pseudomonas aeruginosa* (n = 4), *Staphylococcus aureus* (n = 2), *Proteus mirabilis*

(n = 1)). Five patients (8%) died, in all of whom a virus was detected (influenza A virus (n = 2), RSV (n = 2), coronavirus (n = 1)).

### Diagnostic accuracy

Sixty-two patients were included in the diagnostic accuracy analysis (table 3). Compared to the reference method, SS2R had a sensitivity of 82.9% [95% CI 67.9-92.9] and specificity of 95.2% [95% CI 76.2-99.9] for complete virus detection. In two samples the SS2R initially gave an invalid result (one sample negative, one with rhinovirus in the RT-PCR). After retesting with the SS2R, the results were similar to the reference standard. Discrepant results were found in nine samples. SS2R missed influenza A virus (n = 3), RSV (n = 3) and coronavirus (n = 3) in seven samples. The median Ct-value of 36.04 (SD  $\pm$  4.21)

**Table 2. Virological results of the RT-PCR\* and rapid SS2R\* (n = 62)**

Virus	RT-PCR (n (%) of samples)	SS2R (n (%) of samples)
1 virus - no. (%)	36 (58.1%)	37 (59.7%)
≥ 2 viruses - no. (%)	5 (8.1%) <sup>†</sup>	1 (1.6%) <sup>†</sup>
0 viruses - no. (%)	21 (33.9%)	24 (38.7%)
<b>Pathogens</b>		
Influenza A - no. (%)	22 (35.5%)	19 (30.6%)
RSV - no. (%)	8 (12.9%)	5 (8.1%)
Coronavirus - no. (%)	10 (16.1%)	7 (11.3%)
Rhinovirus - no. (%)	6 (9.7%)	7 (11.3%)
HMPV - no. (%)	1 (1.6%)	1 (1.6%)

HMPV = human metapneumovirus; n = number; no = number; PCR = polymerase chain reaction; RSV = respiratory syncytial virus; SS2R = syndromic sample to result.  
 \*RT-PCR tested: coronavirus, rhinovirus, influenza virus A and B, RSV A and B, human metapneumovirus, adenovirus, bocavirus, enterovirus and parainfluenza virus 1-4. SS2R tested: adenovirus, coronavirus (229E, HKU1, OC43, NL63), human metapneumovirus, rhinovirus, enterovirus, influenza virus A and B, parainfluenza virus 1-4, RSV A and B, and three bacterial pathogens: *Bordetella pertussis*, *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*.  
<sup>†</sup>RT-PCR detected multiple viral pathogens in 5 patients: influenza virus A and RSV (n = 1); influenza virus A and coronavirus (n = 1); RSV and coronavirus (n = 1); coronavirus and rhinovirus (n = 1); and influenza A virus, RSV and coronavirus (n = 1). SS2R detected multiple viral pathogens in one patient: coronavirus and rhinovirus (same patient in which in-house RT-PCR detected coronavirus and rhinovirus)

**Table 3. Sensitivity, specificity, PPV, NPV, accuracy (overall percentage agreement) and kappa of the SS2R compared to RT-PCR (n = 62); diagnostic accuracy is given per sample and per virus**

Concordant result	Sample result SS2R/PCR				Sensitivity % (95%CI)	Specificity % (95%CI)	PPV % (95%CI)	NPV % (95%CI)	Accuracy % (95%CI)	Kappa % (95%CI)
	+/+	+/-	-/+	-/-						
Complete viral panel	34	1	7	20	82.9 (67.9-92.9)	95.2 (76.2-99.9)	97.1 (83.3-99.6)	74.1 (59.1-85.0)	87.1 (76.2-94.3)	0.73 (0.56-0.90)
Influenza A virus	19	0	3	40	86.4 (65.1-97.1)	100 (91.2-100)	100 (79.1-100)	93.0 (82.3-97.5)	95.2 (86.5-99.0)	0.89 (0.77-1.00)
RSV	5	0	3	54	62.5 (24.5-91.5)	100 (93.4-100)	100 (46.3-100)	94.7 (88.0-97.8)	95.2 (86.5-99.0)	0.74 (0.47-1.00)
Coronavirus	7	0	3	52	70.0 (34.8-93.3)	100 (93.2-100)	100 (56.1-100)	94.6 (87.1-97.8)	95.2 (86.5-99.0)	0.80 (0.58-1.00)
Rhinovirus	6	1	0	55	100 (54.1-100)	98.2 (90.5-100)	85.7 (46.2-97.7)	100 (91.9-100)	98.4 (91.3-100)	0.91 (0.75-1.00)
HMPV*	1	0	0	55	100 (2.5-100)	100 (93.5-100)	100 (5.5-100)	100 (91.9-100)	100 (93.6-100)	1.00 (1.00-1.00)

CI = confidence interval; HMPV = human metapneumovirus; kappa = Cohen's kappa coefficient; n = number; no = number; NPV = negative predictive value; PCR = polymerase chain reaction; PPV = positive predictive value; RSV = respiratory syncytial virus; RVP = Respiratory Viral Panel.  
 \*In six patients no RT-PCR result was reported for HMPV

of these discrepant results (n = 9), with one Ct-value > 40 (Influenza A, Ct-value 40.89), was significantly higher than the median Ct-value of 26.03 (SD ± 7.05) of all concordant virus positive results (n = 37), with one Ct-value > 40 (coronavirus, Ct-value 42.23) (p = 0.001). SS2R had a rhinovirus positive result in one sample (RT-PCR sample negative) and an influenza A / influenza B virus equivocal result in one sample (RT-PCR sample influenza A virus,

Ct-value 36.04), the latter being considered concordant in the accuracy analysis.

**Logistics**

The median TAT of the SS2R was 2:06 hours (IQR 1:44-3:16) for patients of whom the SS2R was performed on the day of sampling (n = 46). For 16 of these (35%), results became available during their ED-stay. The median



RT-PCR sample TAT of the 46 patients was 32:00 hours (IQR 26:50-40:42). Based on the questionnaires, laboratory technicians (n = 5) were positive about ease-of-use, short hands-on time ( $\leq 10$  minutes) and fast TAT of the SS2R. A low test capacity, due to the availability of only one SS2R system, resulting in less optimal TATs, was mentioned as a drawback.

## DISCUSSION

This prospective clinical study focuses not only on accuracy and TATs of rapid SS2R testing, but also on applicability and implementation strategies. SS2R showed 90% sensitivity and 95% specificity in the detection of respiratory viruses compared to the current gold standard, RT-PCR. The poor diagnostic accuracy for some viruses, for example RSV, is due to a small number of patients and is reflected in the wide confidence intervals around the accuracy estimates. In our hospital, SS2R had a rapid TAT, even when used in a laboratory setting. The SS2R only had two out of 62 invalid initial results. This SS2R therefore has great potential in the improvement of clinical outcomes

inpatients, for example in terms of targeted oseltamivir prescription and possibly also reduced antibiotic treatment. It may also benefit hospital management, for example by contributing to more adequate use of in-hospital isolation facilities. It should be noted that all swabs were taken during a month in the viral respiratory season with a high prevalence of influenza virus and RSV in particular. Not only the virological results, but also potential effects on clinical outcomes should be viewed from this perspective. Among published articles on the accuracy of the specific SS2R (FilmArray®)<sup>14,35</sup> used in this study, only a few compared SS2R to RT-PCR,<sup>14-20</sup> with a calculated pooled sensitivity of 87.6% [95%CI 84.6-90.1] and specificity of 91.1% [95%CI 87.5-93.7] (n = 945 samples, *table 4*). Differences between studies, including the current study, and the relatively low overall sensitivity might be explained by genetic variability of viruses, differences in sampling and analyzing methods, patient numbers and/or heterogeneity of the patients involved. It is hard to predict genetic variability but methods of sampling, data analysis and patient inclusion are influential. It is therefore useful to find out how accuracy can be optimized before implementing SS2R. First, the sampling site has a

**Table 4.** Overview FilmArray® validation studies with RT-PCR as reference test (n = 7)

Study	Year	FA version	No. samples	Patients	Country	Swab method	Discordant analysis	Sens (%), CI (95%)	Spec (%), CI (95%)	Ref. no.
Pierce <i>et al.</i>	2011	Pre-market version	280	Children 0-22 years	USA	NPA, NPS, EA, BAL, LA	Repeated testing	86.9 (81.4-90.9)	85.1 (73.8-92.2)	12
Hayden <i>et al.</i>	2011	Pre-market version	176	Children 0-18 years	USA	NPS, NPW, BAL, EA	x	90.2 (79.1-95.9)	93.0 (86.3-96.7)	13
Renaud <i>et al.</i>	2012	FDA cleared v1	34	Unclear	USA	NPW, NPS, BAL, EA, SP	Repeated testing	76.5 (58.4-88.6)	x	14
Hammond <i>et al.</i>	2012	Pre-market version	90	Immuno-compromised adults	USA	NPA, BAL	Repeated testing	92.9 (75.0-98.8)	93.5 (83.5-97.9)	15
Van Wesenbeeck <i>et al.</i>	2013	FDA cleared	165	Adults (presenting at GP)	Belgium	NPS	Sequencing	85.1 (75.4-91.5)	100 (94.2-100)	16
Piralla <i>et al.</i>	2014	Pre-market version	72	Neonates < 30 days	Italy	NPA, NPS, BAL, EA	x	100 (92.8-100)	55.6 (22.7-84.7)	17
Andersson <i>et al.</i>	2014	FDA cleared v1.6	128	Children and adults	Sweden	NPS, TS, BAL	Repeated testing	84.2 (75.2-90.4)	77.8 (57.3-90.6)	18

BAL = bronchoalveolar lavage; CI = confidence interval; EA = endotracheal aspirate; *et. al.* = and others; FA = FilmArray®; FDA = Food and Drug Administration; GP = general practitioner; LA = lung autopsy tissue; n = number; no = number; NPA = nasopharyngeal aspirate; NPS = nasopharyngeal swab; NPW = nasopharyngeal wash; PCR = polymerase chain reaction; ref = reference; RT = reverse transcription; RV = respiratory viral; RVP = Respiratory Viral Panel; sens = sensitivity; SP = sputum; spec = specificity; TC = throat swab; USA = United States of America; v = version

significant effect on sensitivity. Although lower respiratory tract samples like bronchoalveolar fluids, have highest sensitivity,<sup>17,28</sup> nasopharyngeal swabs – which were used in the current study – are the most sensitive upper respiratory samples<sup>36,37</sup> and are most feasible in an ED-setting. Second, in analyzing accuracy data for multiple viral pathogens, the number and choice of viruses and the cut-off Ct-value influence accuracy. In this study, accuracy was calculated both per virus and per sample, using initial results and results of repeated testing for invalid results to reflect clinical practice as close as possible. The high cut-off value of > 45 used in this study, leading to a somewhat lower sensitivity of the SS2R due to one influenza A virus (Ct-value of 40.89) that was missed by the SS2R, was chosen to reflect clinical practice, in which higher cut-off values are often used for RNA-viruses. Furthermore, choosing a reference standard, including the performance of a discrepancy analysis, either by repeated testing or by sequencing, greatly influences accuracy. Although RT-PCR is considered the best available reference standard, composite reference standards and discrepancy analyses may lead to higher numbers of agreement. In this study accuracy measures were given in a way the estimates are interpretable by clinicians. From a more epidemiologic point of view, accuracy or percentage agreement (87% [95% CI 76-94]) with Cohen's Kappa statistic (0.73 [95% CI 0.56-0.90]) might be more suitable considering the imperfect reference standard (table 3). Third, significant differences have been shown in the sensitivity of PCR assays between different study populations, with higher sensitivities in patient groups with higher viral loads and lower viral clearance rate<sup>38</sup> due to short symptom duration or specific characteristics such as being a child,<sup>39,40</sup> being immunocompromised<sup>40</sup> or having COPD.<sup>41</sup> The current study is underpowered to compare patient groups, but confirms that discordant SS2R negative results had significantly lower viral loads (e.g. higher Ct-values) than concordant results. In daily practice this means that SS2R has reliable results for patients with high viral loads, being the patients at highest risk of complications and respiratory insufficiency.<sup>42</sup>

In our hospital, SS2R has a rapid TAT compared to that of previous studies (average TAT 2:30 hours (n = 5576)).<sup>34,35,43-48</sup> Since patients were only included during lab opening hours, TATs may not be representative for sample testing during evenings, nights and weekends. A proposal to relocate the SS2R system to the ED raised objections among the staff. Objections included lack of laboratory skills, a highly variable workload of the ED-personnel and need for an isolated test room, making SS2R implementation at the ED unfeasible at our hospital. Also, the ISO-certification would have to be extended for point-of-care testing at the ED. However, the current

study showed that TAT was rapid even when used in a laboratory setting. Apart from TATs, the effect of SS2R testing is also affected by early availability of results, which was delayed in this research setting due to the necessity of asking informed consent and taking a second swab. When swabs are taken shortly upon a patient's arrival at the ED, it can be assumed that the percentage of available SS2R results during ED-stay will be much higher than 35%. Altogether, SS2R implementation in the laboratory setting has great potential in affecting clinical outcomes, with fewer practical issues to overcome than at the ED. The effect of rapid testing can be optimized by extending laboratory opening hours and using more than one SS2R system for parallel testing.

Even though the results of this study regarding diagnostic accuracy, TATs and applicability are promising with respect to clinical outcomes, no exact estimations can be made. A recent randomized controlled diagnostic intervention trial<sup>49</sup> showed beneficial effects of rapid testing with the same SS2R on both oseltamivir prescription, duration of antibiotic treatment and use of in-hospital isolation facilities, strengthening our hypothesis. In this trial, as compared to conventional diagnostics, SS2R led to a 26% increase in correct oseltamivir prescription in influenza virus positive patients (91% versus 65%, p = 0.003) and an 8% decrease in the number of patients who received antibiotic therapy for > 48 hours.<sup>49</sup> However, the percentage of antibiotic prescriptions was similar in both groups and the use of SS2R increased the use of in-hospital isolation facilities for patients with confirmed viral RTI by 8% (17% versus 9%, p = 0.02).<sup>49</sup> The hypothesis that rapid viral SS2R testing in patients with RTI may also reduce antibiotic prescription is one of the most important issues in the current landscape of increasing antibiotic resistance. Nevertheless, since the decision to prescribe antibiotics is not based on the SS2R results alone, neither observational nor experimental studies have so far been able to show an advantageous effect of rapid SS2R testing on antibiotic prescription. The most plausible explanation for this disappointing effect of SS2R testing on antibiotic prescription is that many patients have already been started on antibiotics before the results of a rapid test become available,<sup>49</sup> underlining the importance of an optimal implementation strategy.

In conclusion, rapid syndromic sample to result PCR like the FilmArray<sup>®</sup> Respiratory Panel are fast, easy to use and accurate, especially with high-risk patients. Implementation of rapid mPCR diagnostics in routine care, even when put in a laboratory setting, could further improve clinical management of patients suspected of respiratory tract infection presenting at the emergency department.

## DISCLOSURES

All authors declare no conflict of interest. No funding or financial support was received.

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# Legionella longbeachae; don't miss it!

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## ABSTRACT

We here report on two immunocompetent patients admitted to our hospital within 3 weeks' time, both suffering from pneumonia caused by *Legionella longbeachae* (*L. longbeachae*). The pathogen was identified in broncho-alveolar lavage (BAL) liquid by Polymerase Chain Reaction (PCR), whereas sputum cultures remained negative. This organism is worldwide still relatively unknown and consequently underdiagnosed. However, with an increasing number of confirmed infections in Europe and more specifically in the Netherlands, early awareness and diagnostic measurements are indicated. As routine laboratory techniques like the urine antigen test do not detect *L. longbeachae*, we advocate early use of specific tests for non-pneumophila Legionella species such as PCR. Furthermore, we advocate the start of empirical antibiotic therapy (i.e. ciprofloxacin) and continuation in suspected cases.

## KEYWORDS

ICU, Intensive Care Unit; legionella; *legionella longbeachae*; pneumoniae

## INTRODUCTION

As one of over 50 species, *L. longbeachae* belongs to the extensive family of Legionellae. These gram negative, opportunistic bacteria can cause Pontiac fever and Legionnaires' disease, a multisystem disease including pneumonia with a severe course. It has no particular clinical features that distinguish it from other types of pneumoniae.<sup>4,5</sup> In contrast to *L. pneumophila* which is linked mainly to contaminated water reservoirs, the general source of contamination for *L. longbeachae* is considered to be compost and other growing media.<sup>6,7</sup> Since *L. pneumophila* is responsible for 80-90% of the Legionella infections in Europe,<sup>8-10</sup> our diagnostic system

### What was known on this topic?

*L. longbeachae* is known to be a major cause of Legionellosis in Australia and South East Asia. Over the last decade the incidence of infections by this pathogen has also increased in Europe, including the Netherlands. *L. longbeachae* is not detected by the commonly used urine antigen test, and it often does not grow in diagnostic cultures. Awareness of the risk of non-pneumophila Legionella species is often still limited to immune-deficient patients and severe cases of pneumonia without clinical recovery on empirical antibiotic therapy.

### What does this add?

After the first *L. longbeachae* case report in an immunocompetent patient by Diederer et al. in 2005<sup>1</sup> and recently in a brief photo quiz by Hannivoort et al.,<sup>2</sup> there were, to the best of our knowledge, no other cases published in our country. Since 2014 the national incidence seems on the rise.<sup>3</sup> This case series confirms this observation, and emphasizes the need for early awareness and diagnostic interventions for non-pneumophila legionella species.

is mainly targeting this species. The restricted sensitivity of the urine antigen test and the low sensitivity of culture testing cause the risk of false rejection of the diagnosis Legionella's pneumonia.

*L. longbeachae* is increasingly being reported in Europe.<sup>3,8,9,11</sup> In our local hospital we recently hospitalized two immunocompetent patients within three weeks, both infected by *L. longbeachae*. Given the low incidence of this pathogen, this duo-cluster is remarkable, suggesting an increased presence of *L. longbeachae*. This article emphasizes the importance of early awareness regarding non-pneumophila Legionella infections, and as such advocates the need for targeted PCR usage in hospital laboratories.

**Figure 1.** CT-scans of patient A (panel A) and patient B (panel B) showing typical signs of *Legionella pneumoniae*; extensive infiltration and consolidation with ground glass opacity and areas with cavitation



## CASE REPORTS

Patient A is a 79-year-old man admitted in June 2017 with malaise, fever and heavy diaphoresis. Medical history included type 2 diabetes mellitus and hypertension. He had a history of heavy nicotine abuse in the past with 100 pack years until 8 years ago, implying the possibility of undiagnosed chronic obstructive lung disease. Physical examination showed a heart rate of 80/minute, blood pressure of 200/105 mmHg, SpO<sub>2</sub> of 94% without supplemental oxygen, respiration rate of 16/minute and a tympanic temperature of 40° Celsius. C-reactive protein (CRP) was 197 mg/l and there was minimal leucocytosis. Chest X-ray showed consolidation of the left upper lung. Urine antigen tests (*Streptococcus pneumoniae* and *Legionella pneumophila*) were negative. We suspected pneumonia and started intravenous treatment with cefuroxim. Two days later there was no clinical improvement and CRP rose to 330 mg/l. Sputum cultures and HIV-testing remained negative. On suspicion of atypical bacteria, antibiotics were switched to azithromycin. On day four of admission a BAL was performed, and subsequent PCR on *Legionella* species revealed *L. longbeachae* upon which antibiotics were switched to ciprofloxacin. Despite the positive PCR results, sputum cultures remained negative for *Legionella* species. On day six a chest CT-scan showed extensive bilateral ground glass opacity (figure 1). The following days the patient recovered slowly; respiratory effort, oxygen demand, CRP and leucocyte count decreased. Unexpectedly, he died on the general ward on day ten. Autopsy revealed evident pulmonary oedema, pleural effusion (650 ml bilateral), and cavitation and haemorrhage of the left upper lobe.

The heart was hypertrophic, with signs of subendocardial infarction of the left ventricular cardiac wall, in addition with signs of swelling of liver and spleen implying acute congestive cardiac failure as the probable cause of death.

Patient B, also a 79-year-old man, was admitted three weeks later with dyspnoea, altered consciousness and confusion. Medical history revealed obstructive sleep apnoea syndrome and – uncomplicated – knee surgery 10 days before this current admission. He was a non-smoker. Notable detail was a recent 2-week vacation in Turkey from which he had returned five weeks before this admission. Physical examination on admission showed a heart rate of 104/minute, blood pressure of 130/70 mmHg, SpO<sub>2</sub> of 93% with 6 litres of oxygen through an oral-nasal mask, respiration rate of 30/minute and a tympanic temperature of 37.8° Celsius.

Chest CT-scan showed extensive right-sided ground glass opacity. Urine antigen tests (*Streptococcus pneumoniae* and *Legionella pneumophila*) and sputum cultures remained negative. Because of the recent travel and hence potential atypical pneumonia, treatment with ciprofloxacin and amoxicillin was started. The next day he was transferred to the Intensive Care Unit due to respiratory insufficiency. Physical examination showed tachypnoea of 30/minute, SatO<sub>2</sub> 94% with 10 litres of oxygen per minute on a non-rebreather-mask, tachycardia of 120/minute, blood pressure of 127/71 mmHg and tympanic temperature of 38° Celsius. Laboratory results showed a rise in CRP rise to 318 mg/l and leucocytosis of 25.7 x10<sup>9</sup>/l. Blood gas analysis showed hypoxia (pH 7.41, HCO<sub>3</sub><sup>-</sup> 20.3 mmol/l, Base Excess -3.9 mmol/l, pCO<sub>2</sub> 4.2 kPa, pO<sub>2</sub> 9.2, SatO<sub>2</sub> 94%). He was intubated and mechanically ventilated. The following days his pulmonary condition worsened and on day four

**Figure 2.** Gardening soils are a potential source of contamination for *L. longbeachae*



of admission antibiotics were switched to ceftriaxone. CT-scan showed expansion of infiltration of the left lung (figure 1, panel B) upon which a BAL was performed. On day seven ciprofloxacin was restarted, because clinical suspicion of Legionellosis still remained, and his clinical situation under treatment with ceftriaxone worsened. PCR on atypical pathogens in the BAL liquid tested positive for *L. longbeachae* whereas cultures remained negative. Treatment with ciprofloxacin was continued for three weeks. The patient recovered slowly, and after 18 days he was extubated and eventually discharged from the hospital. Retrospectively, patient A frequently worked with potting soils in his garden, potentially being the source of contamination. Patient B didn't work with compost soils, but he had recently been gardening near his garden pond. Unfortunately the Area Health Authorities performed no source detection investigation, because *L. longbeachae* is not yet recognized as a public health issue.

## DISCUSSION

Despite an increase in the last decade, Legionellosis caused by non-pneumophila species is still rare on our continent. From 2011 to 2015, 3645 culture-confirmed cases of

Legionella's infection in Europe were documented, 83% of which were caused by *L. Pneumophila*.<sup>9,10</sup> Among the non-pneumophila group, *L. longbeachae* is the dominant species with 35 culture-confirmed cases (1%) within these five years.<sup>9-11</sup> In Australia and Eastern Asia *L. longbeachae* is responsible for approximately half of the Legionellosis.<sup>4,12</sup> In the Netherlands we confirmed a maximum of 1 patient per year in the decade preceding 2014, but from then on the incidence appears to rise (table 1). How can this be explained? As we know, many factors contribute to incidence outcomes, such as geographical distribution, infectious dose, infection type, host susceptibility and the sensitivity of detection techniques. A contributory theory could be that there has been a change in the composition of European compost soils. One of the explanations why *L. longbeachae* occurs in large numbers in Australia and South East Asia derives from the theory that the presence of bark containing sawdust and hammer milled bark makes these soils a natural habitat for the bacterium.<sup>12</sup> Nowadays Australian growing materials and soils even come with warning labels attached (figure 2). In Europe, peat rather than composted bark is the major component used in growing media, but the composition is always subject to change.<sup>13</sup>

Another contributing factor could be the wider application of PCR with regard to Legionella species in hospital laboratories nationwide, causing higher detection rates. Formerly incidence rates depended on the availability of appropriate laboratory techniques and skilled employees for analysis. In Scotland, where several outbreaks of *L. longbeachae* were recorded, researchers also described underdetection of these infections.<sup>5,14</sup> Current diagnostics like the urine antigen test are useful to detect *L. pneumophila* serogroup 1, but are not suitable

**Table 1.** Data from the National Institute for Public Health and the Environment (RIVM) showing an increase of incidence in The Netherlands over the past decade<sup>3</sup>

Year	Culture confirmed cases
2008	1
2009	0
2010	1
2011-2013	0
2014	3
2015	2
2016	6
2017	5* (*Accessed 18-10-2017)

for non-pneumophila *Legionella* species.<sup>15</sup> Whereas bacterial culture remains the golden standard for confirmation of *Legionella*'s disease is,<sup>3,10</sup> this technique requires specialized laboratory media as overgrowth with competing flora is a common problem.<sup>5,15</sup> As illustrated by both of our cases the cultures remained 'false' negative, confirming that PCR is an essential test for the diagnosis. In conclusion, *L. longbeachae* is a sporadically emerging pathogen with a severe course of disease. Over the past decade the number of reports on *L. longbeachae* has increased markedly across Europe. Timing of diagnosis and targeted therapy influences the severity and course of the disease. Recommended antibiotic treatment for *L. longbeachae* (like pneumonia with (suspected) atypical bacteria in general and *Legionella* specifically) are fluoroquinolones like ciprofloxacin. Physicians and guideline committees, like the Dutch SWAB-guidelines committee,<sup>16</sup> have adopted the concept of using the 'severity of illness' at the time of clinical presentation (based on validated scoring systems like CURB-65 and Pneumonia Severity Index (PSI), and the need for ICU admission) in order to choose the type of empirical antimicrobial in community acquired pneumonia (CAP). In category IV (severe CAP with ICU admission) it is always recommended to empirically cover *S. pneumoniae*, *Legionella* species and Gram-negative bacteria.<sup>16</sup> It is difficult to predict atypical bacteria being the cause of CAP based on presentation, signs and symptoms of CAP, medical history, physical examination and laboratory results.<sup>17</sup> We recommend extending the medical interview on admission routinely from inquiring after travel abroad and visits to swimming pools and sauna's to questions on recent gardening activity and exposure to gardening soil. We also recommend the early usage of rapid and sensitive PCR techniques on non-pneumophila *Legionella* species in patients with severe and treatment-resistant pneumonia, especially in the presence of risk factors like recent exposure to gardening soils. This way, the diagnostic process may be instrumental in improving things; more awareness among diagnosing clinicians and laboratory staff of non-pneumophila *Legionella* species is necessary to reduce underdiagnoses and associated risks.

## DISCLOSURES

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# A 66-year-old man with hydroxycarbamide induced pneumonitis

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## ABSTRACT

Hydroxycarbamide is used in the treatment of myeloproliferative neoplasms. Hydroxycarbamide is known for its relative lack of severe side effects.

Here we present a 66-year-old man with a severe pneumonitis within three weeks after starting him on hydroxycarbamide. He developed life-threatening respiratory failure and was admitted to an intensive care unit. Extensive testing of blood and cultures from sputum and bronchoalveolar lavage fluid did not reveal a pathogenic microorganism. Discontinuation of the drug and treatment with prednisolone resulted in clinical improvement within 2 days. Radiological resolution was confirmed after one month. The clinical course suggests that the pneumonitis was induced by hydroxycarbamide. We want to alert physicians that, in spite of the common assumption that the use of hydroxycarbamide is relatively safe, patients can develop a severe pneumonitis with detrimental outcome and that hydroxycarbamide should be considered a causative agent in the differential diagnosis of pneumonitis.

## KEYWORDS

Hydroxycarbamide, myeloproliferative neoplasms, side effects, fever, pneumonitis

## INTRODUCTION

Hydroxycarbamide is well absorbed after oral administration, converted to a free radical nitroxide, and transported by diffusion into cells where it inhibits DNA synthesis by inactivating ribonucleotide reductase, resulting in cell death in S phase.<sup>1</sup> Hydroxycarbamide is widely used in myeloproliferative neoplasms. Although there have been concerns about the mutagenic

### What was known on this topic?

Hydroxycarbamide induced pulmonary toxicity is extremely rare. Since 1990 only nine other cases have been described in English literature. Time of onset of hydroxycarbamide pneumonitis after start of treatment can range from two weeks to 12 years.

### What does this add?

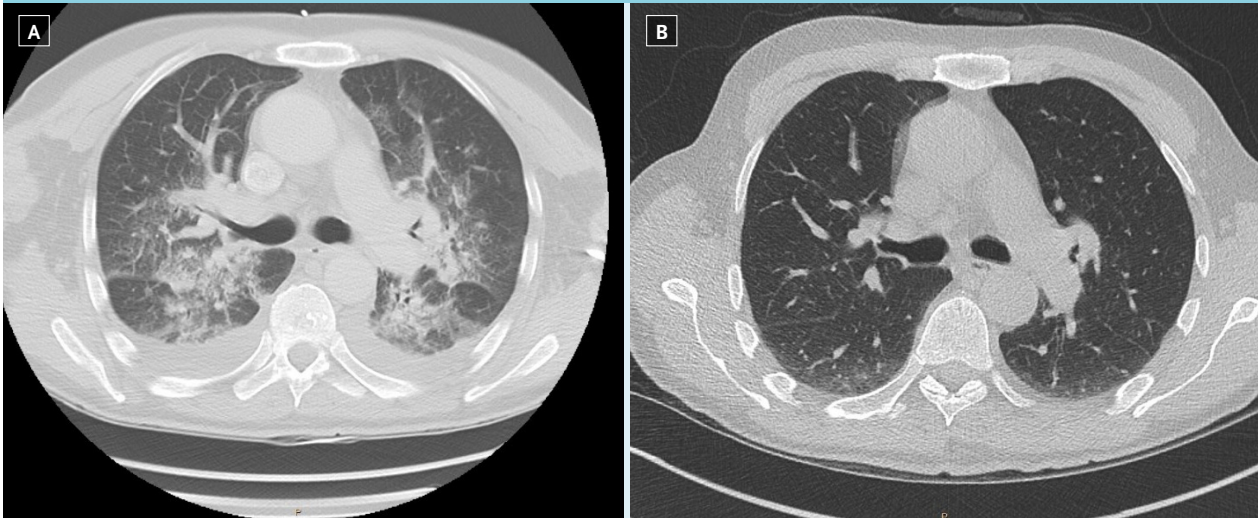
This case report helps heighten clinical awareness of hydroxycarbamide induced pneumonitis. It is the first Dutch case reported in the literature. Since clinical outcome has a wide variability, it is important to recognise the clinical symptoms. Treatment consists of discontinuation of hydroxycarbamide and prednisolone, although the role of the latter treatment is not well established.

and leukemogenic potential of the drug, the risk of development of myeloid malignancies is considered to be lower than with other cytostatic drugs.<sup>2</sup> Side effects include pancytopenia, gastrointestinal discomfort and sometimes chronic mucocutaneous ulcers. We describe a patient who developed life threatening respiratory failure with admission to intensive care unit occurring within three weeks after having been started on hydroxycarbamide.

## CASE

A 66-year-old man with metastasized prostate cancer and myeloproliferative neoplasia (MPN), was admitted to the hospital because of fever. His medical history included appendectomy and prostatectomy for prostate cancer and superficial thrombophlebitis. Five years before admission JAK-2 (50%) MPN was diagnosed based on a platelet count of  $465 \times 10^9/l$  (RW 150-400), elevated haematocrit of 0.51% (RW 0.40-0.50) and normal leucocyte count. Bone marrow

**Figure 1.** CT scan images (axial). Figure 1A. Extensive multifocal consolidations with ground-glass. Pleural fluid is extensive Figure 1B. Three weeks after discontinuation, a significant reduction of pulmonary pathology is depicted



biopsy revealed morphological features compatible with Polycythaemia Vera (PV). Treatment for the prostate cancer consisted of bicalutamide and gosereline.

Because his age of 61 was the only vascular risk factor at the time, he was treated with acetylsalicylate (100 mg/day) and repeated phlebotomies and the initiation of cytoreductive therapy was postponed.

With this treatment haematocrit declined, but platelet counts gradually increased. Because of urinary obstruction a transurethral resection of the bladder neck was planned. In view of the increased risk of postoperative thrombosis due to the combination of hormonal treatment for metastatic prostate cancer, previous thrombophlebitis and JAK-2 driven thrombocytosis ( $700 \times 10^9/l$ ) pre-operative cytoreduction with hydroxycarbamide (500 mg daily) was started.

Nineteen days later he developed a fever and the urologist initiated treatment with ciprofloxacin 500 mg twice a day. Three days later he presented with persistent high fever and was admitted to the urology department and treated with amoxicillin/clavulanate (3 x 1200 mg) in combination with a one-time gift of tobramycin 5 mg/kg.

Next day he complained of dyspnea and fine bilateral pulmonary crackles were heard. The oxygen saturation was 84% (normal > 96%) on breathing room air. Arterial blood gas analysis, on oxygen supply, showed respiratory alkalosis with hypoxemia (pH 7.534;  $pO_2$  7.4 kPa;  $pCO_2$  4.0 kPa; base excess 2.7 mmol/l; bicarbonate 26.7 mmol/l;  $O_2$  saturation 92.1%).

Chest X-ray showed an increase of interstitial fluid (opacity), fluid in fissures and minimal pleural fluid. He received oxygen and furosemide 40 mg intravenously and was admitted to intensive care where he was put on high flow nasal cannula therapy.

Despite a regimen of broad spectrum antibiotics, fever persisted. Cultures from blood, sputum and urine showed no pathogenic microorganisms and urine testing for *Legionella pneumophila* and *Streptococcus pneumoniae* was negative. A polymerase chain reaction (PCR) of a throat swab was negative for Influenza A and B and respiratory syncytial virus (RSV), and there was no serological proof for *Chlamydomphila pneumoniae*, *Chlamydomphila psittaci*, *Legionella pneumophila*, *Mycoplasma pneumoniae* and HIV 1/2. Because of lack of clinical improvement after four days antibiotic treatment was discontinued.

Computed tomography (CT) of the chest revealed extensive multifocal consolidations with ground-glass, bilateral hilar lymphadenopathy and pleural effusion. Examination of fluid obtained by bronchoalveolar lavage fluid showed 40% macrophages, 20.0% neutrophils without malignant cells. Cultures for bacteria, fungi and *M. Tuberculosis* were negative.

Hydroxycarbamide was discontinued and treatment with prednisolone was started in the dosage of 40 mg/day, within two days resulting in rapid clinical improvement. Patient was discharged from hospital one week later in good clinical condition and prednisolone was tapered and stopped after two weeks treatment. A CT scan performed one month after discharge showed significant improvement (figure 1).

## DISCUSSION

We describe a patient with life threatening respiratory failure. No pulmonary pathogens were demonstrated and despite broad spectrum antibiotics his condition deteriorated. There was a dramatic clinical improvement

**Table 1.** A summary of reports regarding hydroxycarbamide induced pneumonitis

Reference	Age	Sex	Disease	Hydroxyurea regimen	Radiodiagnostic findings (CTscan/Xray)	Pathology	Period of onset	Symptoms	Treatment	Outcome
1 Jackson 1990 <sup>8</sup>	66	Male	CML	500 mg / twice daily	Pulmonary consolidation and pleural effusion	NR	3 weeks	Lethargy, weakness and shortness of breath	Withdrawal and steroids	Recovery
2 Hennemann 1992	77	Male	Myeloproliferative syndrome	500 mg / twice daily	Reticulonodular infiltrates	NR	2 weeks	Fever, dyspnea, hypoxemia	Withdrawal and steroids	Recovery
3 Quintas-Cardama <sup>9</sup> 1999	58	Male	Essential thrombocythemia	500 mg / daily	Bilateral interstitial infiltrates and pleural effusion	NR	4 weeks	Fever, hypoxemia	Withdrawal	Recovery
4 Kavuru <sup>10</sup> 1994	78	Female	Myeloproliferative syndrome	500 mg every third day	Peripheral interstitial infiltrates, bilateral pleural effusions	Interstitial fibrosis and hyperplasia of alveolar lining cells	3 months	Dry cough, fever, dyspnea	Withdrawal and steroids	Recovery
5 Sandhu <sup>11</sup> 2000	48	Male	CML	1000 mg 3 times daily (2 weeks) followed by 500 mg / daily	Bilateral interstitial opacities	Interstitial inflammation with poorly formed granulomas	4 weeks	Fever, dyspnea, lethargy, pleuritic chest pain	Withdrawal and steroids	Recovery
6 Wong <sup>12</sup> 2003	63	Male	Chronic idiopathic myelofibrosis	1000 mg / daily	Ground-glass infiltrate	Desquamative interstitial pneumonitis	2 years	Breathlessness and dry cough, afebrile, hypoxic	Withdrawal	Recovery
7 Loo 2007	62	Female	Polycythemia rubra vera	500 mg / daily	Ground-glass infiltrate	Mixed cellular and fibrotic inflammation within the interstitium and granulomas	12 years	Non-productive cough and increasing breathlessness on exertion	Withdrawal	Recovery
8 Internullo <sup>13</sup> 2014	77	Female	Essential thrombocythemia	500 mg / daily	Fine honeycombing at lungbases, ground-glass at the upper lobes	NR	4 years	Severe dyspnea and respiratory failure afebrile	Withdrawal and steroids	Recovery
9 Hisao Imai <sup>14</sup> 2015	84	Male	CMML	500 mg / daily	Ground-glass infiltrate	3 patterns of interstitial pneumoniae; alveolar damage, non-specific interstitial pneumonia, organizing pneumonia	3 months	Breathlessness and a dry cough, afebrile	Withdrawal and steroids	Dead
10 recent	66	Male	Essential thrombocythemia	500mg/ daily	Ground-glass infiltrate, extensive pleural fluid	NR	3 weeks	Fever, respiratory distress	Withdrawal and steroids	Recovery

NR: not reported

after discontinuation of hydroxycarbamide and treatment with prednisolone. The clinical course in combination with the radiologic findings is compatible with a drug-induced pneumonitis. Antineoplastic drugs such as busulfan, bleomycine, chlorambucil, cyclophosphamide, cytosine arabinoside, melphalan, methotrexate, mitomycin and procarbazine have been associated with severe pulmonary toxicity.<sup>3,4</sup> Hydroxycarbamide induced pulmonary toxicity is rare as only nine other cases have been described in the English literature (*table 1*) since 1990. Time of onset of hydroxycarbamide associated pneumonitis after initiation of treatment is highly variable, ranging from two weeks<sup>5</sup> to 12 years.<sup>6</sup> In this patient the respiratory symptoms occurred on the twentieth day after the start of the treatment.

The severity of the clinical symptoms reported is variable. Treatment is not established. Three out of nine patients recovered upon withdrawal of hydroxycarbamide alone. The other six were treated with corticosteroids in dosages ranging from 40 mg to 1000 mg, as in our patient.<sup>5</sup> Most patients recovered, one patient died.

Drug-induced pneumonitis is a diagnosis per exclusionem. A drug lymphocyte stimulation test (DLST) has been introduced as a diagnostic test but its specificity is limited, since a DLST often yields false-positive results.<sup>7</sup> Since the severity of the side effect a rechallenge is not mandatory. We would like to emphasize that, despite the common assumption that the use of hydroxycarbamide is relatively safe, patients can develop a severe pneumonitis with detrimental outcome and that hydroxycarbamide should be considered a causative agent in the differential diagnosis of pneumonitis.

## DISCLOSURES

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# Sudden subcutaneous emphysema and dysphagia in a 46-year-old woman

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## CASE REPORT

A 46-year-old woman presented at the emergency department with progressive swelling of the neck, acute dysphagia, altered voice and retrosternal pain after choking that morning. While swallowing muesli she felt a sharp pain in her throat, followed by some mild coughing. Her medical history was not contributory, but one of her sisters had died of esophageal carcinoma.

Direct flexible laryngoscopy by the otorhinolaryngologist did not show abnormalities, particularly supraglottic swelling or a foreign body. Preventive endotracheal intubation was performed because of a threatened airway. CT scan of the neck and chest identified diffuse subcutaneous emphysema, and extensive pneumomediastinum, as shown in *figure 1*. No injuries of the esophagus or trachea were noticed on the CT scan: no pneumothorax, free intra-abdominal gas or foreign body.

## WHAT IS YOUR DIAGNOSIS?

See page 303 for the answer to this photo quiz.

**Figure 1.** CT-scan of the neck and chest, showing diffuse subcutaneous emphysema of the neck and pneumomediastinum



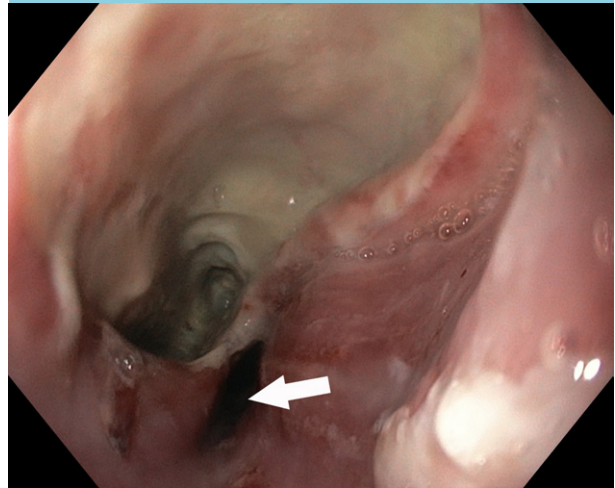
**DIAGNOSIS**

Endoscopy showed a 9 cm rupture of the proximal esophagus (*figure 2*), macroscopically not suspect for underlying malignant or ulcerative disease. Broad spectrum antibiotics were started, and the patient was transferred to a tertiary academic center, where a 12 cm partially covered esophageal stent was placed. Despite antibiotic treatment, mediastinal and pleural drainage, her condition worsened and she developed mediastinitis. CT-thorax showed progressive infiltration, increase in pleural effusion and a persisting mediastinal pocket. Ultimately a video assisted thoracoscopy (VATS) was performed and two additional drains were placed. After two weeks the stent was removed because of a persistent defect not covered by the stent. This persistent defect spontaneously closed within the subsequent seven days.

Subsequently, she gradually recovered. A video fluoroscopic swallowing exam did not show any leakage. Oral feeding was being built up slowly, and one month after hospital admission she was discharged in good condition.

Esophageal perforation is a rare but potentially life-threatening condition, with overall mortality of 18%.<sup>1</sup> Spontaneous perforation known as Boerhaave's syndrome accounts for approximately 15% of perforations.<sup>2</sup> Inconsistently with the classic distal perforation of this syndrome, our patient presented with a spontaneous proximal rupture, most likely due to the muesli or a sharp foreign body in the muesli, although we never retrieved one.

**Figure 2.** Endoscopy showing the native esophagus lumen (white arrow), with next to it a large 9 cm defect of the wall of the proximal esophagus

**DISCLOSURES**

All authors declare no conflict of interest. No funding or financial support was received.

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# Petechiae on the heart

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## CASE

A 41-year-old woman with a history of hypertension and endometriosis presented at the emergency department (ED) with hyperglycaemia. The day of her presentation she had been seen in the preoperative department, being examined in relation to a planned operation. There were no abnormalities.

We saw a moderately ill woman in the ED with hypertension, tachycardia and a blood glucose of 24.3 mmol/l. She had a short history of vomiting and diarrhea. About 45 minutes later she was in a state of confusion and fear, with progressive tachycardia, tachypnea and hypertension, and exhibiting a pale, marbled skin with barely palpable peripheral pulsations. Strikingly, there were now petechiae. Laboratory analysis showed signs of hemolysis, leucocytosis, deep thrombocytopenia and acute kidney failure.

The patient rapidly deteriorated with progressive circulatory and respiratory failure. Treatment was started with 1200 mg Augmentin/300 mg Gentamicin, fluid challenges and hydrocortisone 100 mg. Catheterisation showed dark, rusty coloured urine.

Despite treatment a CPR setting developed. Cardiac ultrasound showed inferior wall movement abnormalities suggesting myocardial infarction, blood results showed high cardiac enzymes. Despite resuscitation and maximal supportive therapy the patient died.

Figure 1. Petechiae on the heart



Obduction showed extensive bleeding foci and thrombi in multiple blood vessels including the myocardium and the kidneys (*figure 1*).

## WHAT IS YOUR DIAGNOSIS?

See page 305 for the answer to this photo quiz.

**DIAGNOSIS**

Postmortem evaluation shows an ADAMTS<sub>13</sub> activity of only 2% (normal 30-200) and autoantibodies against vWF protease, confirming one of our originally suspected diagnoses: Thrombotic Thrombocytopenic Purpura (TTP). It was presumed that extensive clotting and hemorrhagic changes in the myocardium were the principal cause of death.

TTP is an acute life-threatening illness with thrombosis in small blood vessels. TTP is an illness of all ages, mostly seen in adults, and in about 75% of cases in women.<sup>1</sup> The majority of cases are based on auto-immune antibodies against an inhibitory factor of blood clotting (the ADAMTS-13). With the resulting intravascular clotting there is an immense consumption of thrombocytes, and hemolysis develops due to mechanical damage of red blood cells, among other things leading to the occurrence of schistocytes. Multi-organ failure develops, petechiae and purpura can arise, either spontaneous or after only minor trauma because of the deep thrombocytopenia.

We initially suspected that the diarrhea in this case might be caused by a shiga toxin producing bacterium, causing

a hemolytic uremic syndrome. Post-mortem feces analysis did not confirm this suspicion. Whether the diarrhea was related to another type of infection or caused by gut wall damage through thrombotic complications due to the ADAMTS<sub>13</sub> deficiency remains open for discussion.

Untreated TTP has an estimated mortality of 90%.<sup>1</sup> Under normal circumstances, treatment consists of plasma-exchange (PEX), which provides functioning ADAMTS<sub>13</sub>; such treatment should be started immediately. When PEX cannot be started, plasma transfusion can be considered. Presentation with the classic five symptoms (microangiopathic hemolytic anemia, thrombocytopenia, fever, acute kidney failure and neurological symptoms) probably occurs in only 5% of cases.<sup>1</sup>

Thanks to: dr. J.E. Boers, pathologist

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# Massive lymphadenopathy in a patient with human immunodeficiency virus infection

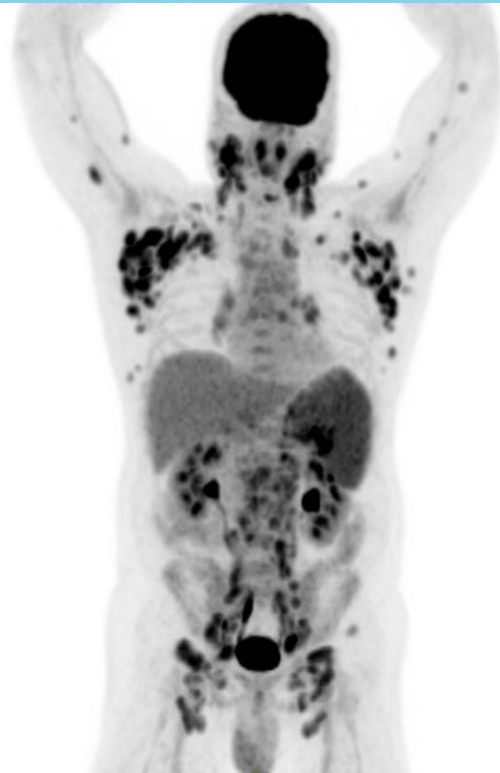
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## CASE REPORT

A 54-year-old man, originally from Sri Lanka, was admitted to our hospital with fever, night sweats and enlarged axillary lymph nodes. His medical history included human immunodeficiency virus (HIV) infection, diagnosed three years prior to presentation, with a CD4 count of 27/uL (CDC C3), primary syphilis, candida esophagitis, cytomegalovirus retinitis and psychosis. Medication consisted of emtricitabin, tenofoviridisoproxil, duranavir and ritonavir. Six months before presentation medication had been changed to emtricitabin, tenofoviridisoproxil, elvitegravir and cobicistat, after which CD4 count rose from below 100/ $\mu$ L to above 200/ $\mu$ L. For the past two months he had been complaining of tiredness, coughing and fever. Physical examination revealed no abnormalities other than enlarged axillary and cervical lymph nodes. Laboratory investigation showed a C-reactive protein level of 70 mg/l and a chest X-ray was unremarkable. His CD4 count and viral load were 220/ $\mu$ L and 20 copies/ml, respectively. A  $^{18}$ F-fluorodeoxy-glucose positron emission tomography (FDG PET) combined with a computed tomography (CT) scan was performed (*figure 1*).

**Figure 1.**  $^{18}$ F-fluorodeoxy-glucose (FDG) positron emission tomography/computed tomography showing generalized extensive lymphadenopathy and splenomegaly with high avidity for FDG



## WHAT IS YOUR DIAGNOSIS?

See page 307 for the answer to this photo quiz.

## ANSWER TO PHOTO QUIZ (PAGE 306)

## MASSIVE LYMPHADENOPATHY IN A PATIENT WITH HUMAN IMMUNODEFICIENCY VIRUS INFECTION

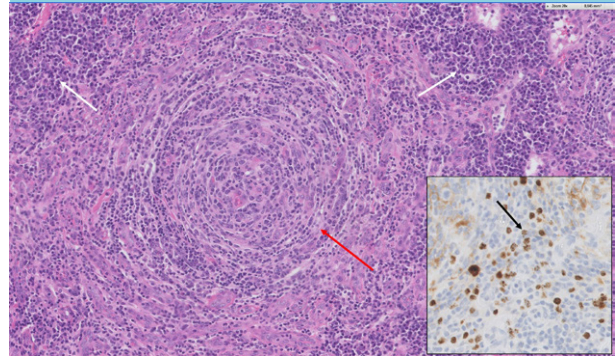
## DIAGNOSIS

The FDG PET/CT scan shows extensive lymphadenopathy and splenomegaly suggestive for multicentric Castleman's disease (MCD), although malignant lymphoma, opportunistic infections and sarcoidosis cannot be excluded. Histology and immunohistochemistry of an excised axillary lymph node confirmed the diagnosis of MCD and immunostaining for human herpesvirus-8 (HHV-8) on biopsy was positive (figure 2).

MCD is a recurring and remitting lymphoproliferative disorder with an incidence in HIV patients of around 8.3 per 10,000 patient-years, in which it is associated with HHV-8.<sup>1</sup> The pathogenesis is related to infection of HHV-8 in immunoblasts and the production of viral interleukin-6 (IL6), but the exact mechanism is unknown. CD4 counts and viral load at presentation can vary and are not associated with the risk of developing MCD. Patients typically present with fever and lymphadenopathy. Splenomegaly, hepatomegaly and fluid retention are also common findings and concomitant Kaposi sarcoma is found in approximately 70% of the patients.<sup>2</sup> Histologically, pathological variants of lymph node biopsies can be divided into three categories: The plasma cell variant and the mixed variant each represent approximately half of cases, the hyaline vascular variant represents 4%. HHV-8 testing on biopsy or serum is positive in 98% of cases associated with HIV. Diagnosis is made when clinical criteria as described by the French *Agence Nationale de Recherche sur le SIDA 117 CastlemaB* trial group are met, in combination with histological findings.<sup>1,2,3</sup>

Evidence on the treatment for MCD is of low quality, current expert opinion is to treat with rituximab. Multiple studies have combined rituximab with antiviral therapy or with chemotherapy, but evidence is of low quality due to small study populations. Anti-IL6(R) therapy shows promise as a second line regimen, but is still under investigation. Antiretroviral therapy (ART) needs to be initiated in newly diagnosed HIV patients and optimized in existing ones already on ART. However, MCD can also be provoked by a rise in CD4 count in the context of an immune reconstitution inflammatory syndrome, as could have been the case in our patient.<sup>2</sup> All-cause mortality for HIV patients with MCD is high, around 25% with a median follow-up of 27 months.<sup>1,3</sup> Imaging is not always necessary for diagnosis but an FDG PET/CT-scan can

**Figure 2.** Lymph node involved with multicentric Castleman's disease. The larger image shows a hematoxylin and eosin stain with in the follicle regression of the germinal center with increased vascularity and concentric arrangement of lymphocytes ('onion skin' pattern) (red arrow). In the interfollicular areas large sheets of plasma cells (white arrows). In the inserted picture human herpesvirus-8 (HHV-8) positive cells in the mantle zones with a finely stippled nuclear staining (black arrow)



assist in distinguishing from concurrent conditions, can aid in choosing a lymph node to excise and can confirm complete remission after initial treatment.<sup>4</sup> Our patient was treated with rituximab and valganciclovir for four weeks and showed complete remission on a follow-up FDG PET/CT-scan three months later.

## ACKNOWLEDGEMENTS

We thank dr. L.B. Meijer-Jorna for disclosure of imaging of histology and immunohistochemistry.

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# A nuclear reactor: more than the producer of energy and radionuclides

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## KEYWORDS

Nuclear research reactor, instrumental neutron activation analysis, enriched stable isotopes, radionuclides

*Dear editor,*

Nuclear reactors are usually associated with the production of energy. However, some reactors provide radionuclides, now widely applied in clinical medicine and research. Both  $\alpha$  and  $\beta$  emitters can be effective in the treatment of tumors and metastases, while  $\gamma$ -emission allows imaging of organs and activity of biological processes. A less well known application of a research reactor is instrumental neutron activation analysis (INAA), a technique for qualitative and quantitative multi-element analysis of major, rare and trace elements in all kinds of materials including human tissues, nails, hair and blood. The method is based on the bombardment of a sample with neutrons followed by capture of a neutron by the nucleus of an isotope of an element and subsequent conversion to a radioactive isotope.<sup>1</sup> The radioactive emission characteristics and decay paths of the various isotopes are well known and this way the presence and concentration of more than 50 elements can be measured. Using INAA it could recently be shown that in contrast to Dutch patients, the majority of Sudanese iron deficient patients also have zinc deficiency.<sup>2</sup> In contrast to other techniques, which measure on an atomic level, INAA is not restricted to measurement in small samples, it can even be used to analyse samples weighing several kilograms. This is especially of importance when an element is not distributed homogeneously in materials, as can be the case in food, but also in human

tissues. INAA is now actively used in biomonitoring, the measurement of the body burden of toxic chemical compounds and elements in biological substances. Lichens in particular have proven useful in registering pollution in the proximity of industries, as well as in measuring elements transported by air masses over large distances.<sup>3</sup> A promising development is the use of enriched stable isotopes, an attractive alternative for the application of radioactive tracers in the study of the bioavailability and distribution of essential trace elements and metals in the human body. The enriched stable isotope Fe<sup>58</sup> has been used, for example, to study iron metabolism in vivo in patients with iron deficiency and hemochromatosis, avoiding the radioactive side effect of the formerly used Fe<sup>59</sup> isotope.<sup>4</sup> Since the number of research reactors is limited, access to its facilities can be problematic. However, with the focus of the reactor of the TU Delft on health and environment, clinical investigators are encouraged to explore its potentials.

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