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Halbeisen, Florian S.; Shoemark, Amelia; Barbato, Angelo; Boon, Mieke; Carr, Siobhan; Crowley, Suzanne

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# Time trends in diagnostic testing for PCD in Europe

Halbeisen Florian S<sup>1</sup>, Shoemark Amelia<sup>2-3</sup>, Barbato Angelo<sup>4-5</sup>, Boon Mieke<sup>6</sup>, Carr Siobhan<sup>2</sup>, Crowley Suzanne<sup>7</sup>, Hirst Rob<sup>8</sup>, Karadag Bulent<sup>9</sup>, Koerner-Rettberg Cordula<sup>10</sup>, Loebinger Michael R<sup>11</sup>, Lucas Jane S<sup>12</sup>, Maitre Bernard<sup>13-14</sup>, Mazurek Henryk<sup>15</sup>, Özçelik Uğur<sup>16</sup>, Martinů Vendula<sup>17</sup>, Schwerk Nicolaus<sup>18</sup>, Thouvenin Guillaume<sup>13,19-20</sup>, Tschanz Stefan A<sup>21-22</sup>, Yiallourous Panayiotis<sup>23</sup>, \*Goutaki Myrofora<sup>1,24</sup>, \*Kuehni Claudia E<sup>1,24</sup>

\* Both authors contributed equally

## Author affiliations:

1. Institute of Social and Preventive Medicine, University of Bern, Switzerland
2. Department of Paediatrics, Primary Ciliary Dyskinesia Centre, Royal Brompton and Harefield Foundation Trust, London, UK
3. School of Medicine, University of Dundee, Dundee, Scotland
4. for the Italian PCD Consortium
5. Department of Paediatrics, University of Padova, Padova, Italy
6. Department of Paediatrics, University Hospital Gasthuisberg, Leuven, Belgium
7. Unit for Paediatric Heart, Lung, Allergic Diseases, Rikshospitalet, Oslo, Norway
8. Department of Infection, Immunity and Inflammation, Institute for Lung Health, University of Leicester, Leicester, United Kingdom
9. Department of Pediatric Pulmonology, Marmara University, School of Medicine, Istanbul, Turkey
10. Department of Paediatric Pneumology, University Children's Hospital of Ruhr University Bochum, Germany
11. Host Defence Unit, Royal Brompton and Harefield NHS Foundation Trust, London, UK
12. Primary Ciliary Dyskinesia Centre, NIHR Respiratory Biomedical Research Centre, University of Southampton, UK
13. on behalf of the French Reference Centre for Rare Lung Diseases
14. Hopital intercommunal de Créteil, Service de Pneumologie, DHU ATVB, Université Paris Est Créteil, France
15. Department of Pneumology and Cystic Fibrosis, Institute of Tuberculosis and Lung Disorders, ul.Prof.Rudnika 3b, 34-700 Rabka - Zdrój, Poland
16. Department of Pediatric Pulmonology, Hacettepe University Faculty of Medicine, Ankara, Turkey
17. Pediatric Department, Charles University Prague and University Hospital Motol, Prague, Czech Republic.
18. Clinic for paediatric pulmonology, allergiology and neonatology, Hannover Medical School, Germany
19. Paediatric Pulmonary Department, Trousseau Hospital APHP, Sorbonne Universities and Pierre et Marie Curie University, Paris, France
20. INSERM U938-CRSA, Paris, France
21. on behalf of the Swiss PCD Group
22. Institute of Anatomy, University of Bern, Bern, Switzerland
23. Medical School, University of Cyprus, Nicosia, Cyprus
24. Paediatric Respiratory Medicine, Children's University Hospital of Bern, University of Bern, Switzerland

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3 47 *To the Editor:*

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5 48 Despite recent advances in diagnostic methods, diagnosis of primary ciliary dyskinesia (PCD)  
6 49 remains complex. We need a combination of different diagnostic tests, and all have their limitations  
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8 50 [1]. In 2009, the first European Respiratory Society Task Force (ERS TF) on PCD in children  
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10 51 published recommendations [2], suggesting that: 1) Nasal nitric oxide (nNO) should be measured  
11 52 to screen for PCD in patients aged  $\geq 5$  years [3]; and 2) video microscopy (VM) analysis of ciliary  
12  
13 53 beat pattern and frequency [4] plus electron microscopy (EM) [5] should be the key confirmatory  
14 54 diagnostic tests. Genetic testing was not recommended as part of the initial diagnostic testing, but  
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16 55 as additional test for inconclusive cases. The recommended test combination was nNO, VM and  
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18 56 EM for patients aged  $\geq 5$  years and VM plus EM for younger patients.

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20 57 In 2017, a second ERS TF on PCD diagnosis revised the accumulated literature and published  
21 58 evidence-based guidelines [6]. Although evidence-based guidelines have become the norm in  
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23 59 research, their practical implementation can be challenging [7]. We wanted to assess whether the  
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25 60 2009 diagnostic recommendations had been implemented and how diagnosis of PCD changed in  
26 61 Europe over time. This knowledge will help to improve implementation of the new guidelines.

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28 62 We analysed data from the international PCD cohort (iPCD) (details are published elsewhere [8]).  
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30 63 By May 2018, iPCD included data on 3733 patients from 26 centres in 21 countries. For this study,  
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32 64 we included all datasets from European centres that tested patients with PCD, both before and  
33 65 after 2009, and had complete information on nNO, EM and VM testing. We excluded patients in  
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35 66 whom diagnosis was based only on clinical presentation, patients with unknown dates of testing.  
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37 67 We included 2108 patients from 16 centres (11 European countries) (Belgium, Cyprus, Czech  
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39 68 Republic, France, Germany, Italy, Norway, Poland, Switzerland, Turkey and United Kingdom); 51%  
40 69 were male, 818 patients (39%) had been diagnosed before and 1290 after 2009. All three  
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42 70 recommended tests were available in all countries, with the exception of Norway where VM testing  
43 71 was not available neither before nor after 2009.

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46 72 Based on the 2009 recommendations, we only considered nNO measurements in patients aged 5  
47 73 years or older [2]. We considered the nNO test as positive when nNO was below  $77 \text{ nL}\cdot\text{min}^{-1}$   
48  
49 74 [9,10]. VM had been performed with different techniques over time, with high speed video analysis  
50 75 being the most commonly used technique in recent years. We classified VM and EM results as  
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52 76 pathological based on information provided by the centres on the beat frequency, beat pattern and  
53 77 cilia ultrastructure. For each patient, we defined the calendar year of diagnosis based on the date  
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55 78 of the earliest positive test result. We then assessed whether there was a change over time in the  
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57 79 proportion of diagnosed patients who had received a) the recommended test combination; b) any  
58 80 single test. We compared the proportion of patients with the recommended test combination (VM  
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81 and EM for patients aged <5 years and nNO, VM and EM for older patients) for the two time  
82 periods, before and after 2009. We used R version 3.1.2 for all analyses.

83 **Recommended test combination:** Overall, we found no significant trend over time in the use of  
84 the test combination. The three tests had been used in 54% of patients diagnosed before 2009 and  
85 in 57% after 2009 ( $p=0.15$ ) (Figure 1). In preschool children the proportion diagnosed with the  
86 recommended combination was 72% before and 75% ( $p=0.47$ ) after 2009; in older patients it  
87 increased from 46% to 52% ( $p=0.03$ ). Results differed between countries. Few countries (e.g.  
88 Belgium, Cyprus) combined all 3 tests already before 2009 for most patients and continued to do  
89 so after 2009. In Germany, the UK and the Czech Republic, the combined use of all 3 tests was  
90 common already before 2009 but increased even more after 2009, with almost  $\frac{3}{4}$  of the patients  
91 tested according to recommendations. The remaining countries (Turkey, Switzerland, Italy, France  
92 and Poland) showed little or no change over time. In these countries, less than half of the patients,  
93 were tested with all 3 approaches even in the later period.

94 **Nasal NO** testing increased overall from 63% before 2009 to 84% afterwards ( $p<0.001$ ). This  
95 increase was seen in most countries (Figure 1). After 2009, nNO was measured in over  $\frac{3}{4}$  of  
96 patients in all countries, except in Czech Republic (65%), Italy (70%) and UK (77%).

97 **Electron microscopy** was frequently performed before 2009 (97%) but decreased to 80%  
98 ( $p<0.001$ ) in the later period. Its use became less common in Poland (79% to 69%), Switzerland  
99 (88% to 62%) and Turkey (100% to 18%), in all other countries it remained stable or increased  
100 after 2009. **Video microscopy** analysis increased overall from 76% to 87% ( $p<0.001$ ). This was  
101 mainly because the use of VM for PCD diagnosis increased considerably in Italy (36% to 69%) and  
102 Turkey (25% to 88%). In most countries, its use remained stable, while in Switzerland (50% to  
103 21%) it decreased substantially.

104 This is the first multi-national study that compared diagnostic testing in PCD patients between  
105 countries and over time. Although a large number of countries contribute to iPCD, some had to be  
106 excluded for this analysis as they only contributed patients diagnosed after 2009 to the iPCD  
107 cohort. Thus, our study describes how the consensus recommendations were implemented in 11  
108 countries. They are not representative for all European countries, but only for those with  
109 established PCD diagnostic protocols. In this analysis we included both children and adults.

110 However when we limited the analysis to children only, for whom the 2009 recommendations were  
111 intended, results remained similar.

112 Our results suggest that the implementation of the recommended diagnostic combination of nNO,  
113 EM and VM testing after the 2009 consensus statement remained low. This reflects the complex  
114 nature of PCD diagnostics and the regional resources. Many countries continued to perform only  
115 one or two of the recommended tests. There are several explanations for this observation. First,

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3 116 the availability of local resources could have led to the development of alternative diagnostic  
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5 117 pathways, which may have been most appropriate for the local situation at that time. All PCD  
6 118 diagnostic tests need specialised expensive equipment and personnel experienced in analysis of  
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8 119 VM and EM results, which are not available in all settings. Limited resources or decentralised  
9 120 healthcare might not have allowed to set up diagnostic centres with scientists experienced in all  
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11 121 methods. For countries with limited resources cost-effective alternatives for diagnostic testing have  
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13 122 been suggested, which might provide an acceptable diagnostic accuracy [11]. Second, since 2009  
14 123 the use of other methods including genetic testing [12,13] and immunofluorescence microscopy  
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16 124 [14,15] became more widespread. These newer methods might have been used instead of the  
17 125 recommended tests in some centres. Lastly, the lack of sufficient evidence supporting the use of  
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19 126 some diagnostic tests in 2009 might have prevented some countries to implement the full set of  
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21 127 recommended tests but let them to develop their own diagnostic algorithms. We found  
22 128 considerable heterogeneity between countries in the use of the three tests. Overall, countries with  
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24 129 low prior use of nNO showed improvement and nNO is now used in most patients aged  $\geq 5$  years  
25 130 suspected for PCD. For the proportion of patients who were still not tested after 2009, we  
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27 131 speculate that nNO was not performed as a screening test, and the primary investigators chose to  
28 132 do directly one or both of the other tests. In this case, if the diagnosis was already established  
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30 133 based on the results of the other tests, the patients might not have been invited posthoc to perform  
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32 134 also nNO measurement. This would be in line with the recommendations. We found that use of EM  
33 135 analysis decreased, and VM increased, suggesting that there might be a shift from EM to VM  
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35 136 overall. Possible reasons are the realization that a significant proportion of patients have normal  
36 137 EM findings [16] and the high costs of EM analysis combined with an increased availability of VM,  
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38 138 so that only patients with inconclusive VM results were referred for EM testing. The overall  
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40 139 changes in use of VM and EM analyses were strongly affected by the marked increase in VM and  
41 140 decrease in EM analysis in Turkish patients. This shift is explained by the development of a new  
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43 141 PCD centre, which uses VM more and EM less frequently.  
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45 142 The 2009 PCD diagnostic consensus is a typical example of how difficult it is to implement  
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47 143 guidelines in clinical practice. Even though the recommendations were widely presented in  
48 144 scientific conferences and meetings, improving knowledge is not sufficient to change daily  
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50 145 practices. A synthesis of systematic reviews on clinical guideline implementation strategies showed  
51 146 that passive dissemination was an ineffective measure and that implementation strategies should  
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53 147 be multifaceted, and actively engage clinicians throughout the process [7]. In the case of PCD  
54 148 diagnosis, implementation is further hindered by fragmentation of national diagnostic services in  
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56 149 many centres and the cost of diagnostic equipment. In our study, countries with limited resources  
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58 150 (e.g. Poland, Turkey) or decentralised diagnosis (e.g. France, Italy, Switzerland) performed the  
59 151 recommended test combination less frequently, than countries with more resources (e.g. Germany,  
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152 Belgium, UK) or established centralised PCD diagnosis (e.g. Cyprus, UK). National and multi-

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153 national collaborations, such as the European Reference Network for respiratory diseases (ERN-  
Lung; <https://ern-lung.eu/>) might play an important role, in the future to facilitate centralised  
diagnosis and standardised patient care. With the further development and improvement of  
diagnostic tests for PCD and with new centres emerging, that might lack the necessary expertise,  
there is an increased need for national and international collaboration in PCD diagnostic testing.

158 Overall, we found a low adherence to the 2009 consensus recommendations mainly due to the  
159 decrease in use of EM analysis in some countries. This resulted in low use of the recommended  
test combination. To further improve PCD diagnosis, we must be more diligent and engaging in  
implementing the new evidence-based guidelines published in 2017, putting more emphasis on  
establishing specialised diagnostic centres and close international collaboration.

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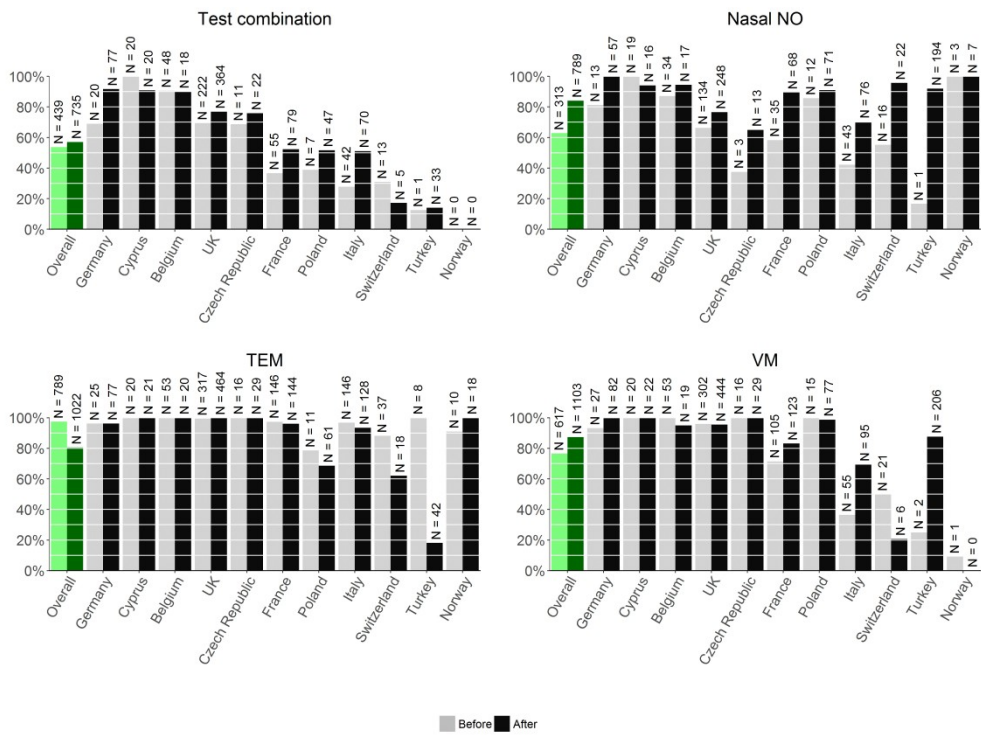


Figure 1: Proportion of performed diagnostic tests in European countries before and after the 2009 consensus statement on PCD diagnostics.