and declining costs of DNA sequencing have improved the accuracy and availability of metagenomic technology. To bring this approach into routine clinical practice, however, additional work is imperative: establishing microbiome-based diagnosis standard by integrating clinically relevant technologies, optimizing protocol to reduce variability and shorten the time in specimen preparation, and developing a platform for automated DNA extraction, genomic sequencing, and result interpretation. Nevertheless, the metagenomic technology holds great promise for identification of pathogens, real-time monitoring of microbial communities, and reducing infection and mortality of patients. ■

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References

- Melsen WG, Rovers MM, Groenwold RH, Bergmans DC, Camus C, Bauer TT, Hanisch EW, Klarin B, Koeman M, Krueger WA, *et al*. Attributable mortality of ventilator-associated pneumonia: a meta-analysis of individual patient data from randomised prevention studies. *Lancet Infect Dis* 2013;13:665–671.
- Chastre J, Trouillet JL, Combes A, Luyt CE. Diagnostic techniques and procedures for establishing the microbial etiology of ventilator-associated pneumonia for clinical trials: the pros for quantitative cultures. *Clin Infect Dis* 2010;51:S88–S92.
- Mentec H, May-Michelangeli L, Rabbat A, Varon E, Le Turdu F, Bleichner G. Blind and bronchoscopic sampling methods in suspected ventilator-associated pneumonia: a multicentre prospective study. *Intensive Care Med* 2004;30:1319–1326.
- Shorr AF, Sherner JH, Jackson WL, Kollef MH. Invasive approaches to the diagnosis of ventilator-associated pneumonia: a meta-analysis. *Crit Care Med* 2005;33:46–53.
- Douglas IS, Price CS, Overdier KH, Wolken RF, Metzger SW, Hance KR, Howson DC. Rapid automated microscopy for microbiological surveillance of ventilator-associated pneumonia. *Am J Respir Crit Care Med* 2015;191:566–573.
- Wilson MR, Naccache SN, Samayoa E, Biagtan M, Bashir H, Yu G, Salamat SM, Somasekar S, Federman S, Miller S, *et al*. Actionable diagnosis of neuroleptospirosis by next-generation sequencing. *N Engl J Med* 2014;370:2408–2417.
- Doughty EL, Sergeant MJ, Adetifa I, Antonio M, Pallen MJ. Culture-independent detection and characterisation of *Mycobacterium tuberculosis* and *M. africanum* in sputum samples using shotgun metagenomics on a benchtop sequencer. *PeerJ* 2014;2:e585.
- American Thoracic Society; Infectious Diseases Society of America. Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. *Am J Respir Crit Care Med* 2005;171:388–416.
- Stoesser N, Batty EM, Eyre DW, Morgan M, Wyllie DH, Del Ojo Elias C, Johnson JR, Walker AS, Peto TE, Crook DW. Predicting antimicrobial susceptibilities for *Escherichia coli* and *Klebsiella pneumoniae* isolates using whole genomic sequence data. *J Antimicrob Chemother* 2013;68:2234–2244.
- Rohde H, Qin J, Cui Y, Li D, Loman NJ, Hentschke M, Chen W, Pu F, Peng Y, Li J, *et al*. E. coli O104:H4 Genome Analysis Crowd-Sourcing Consortium. Open-source genomic analysis of Shiga-toxin-producing *E. coli* O104:H4. *N Engl J Med* 2011;365:718–724.
- Snitkin ES, Zelazny AM, Thomas PJ, Stock F, Henderson DK, Palmore TN, Segre JA; NISC Comparative Sequencing Program Group. Tracking a hospital outbreak of carbapenem-resistant *Klebsiella pneumoniae* with whole-genome sequencing. *Sci Transl Med* 2012;4:148ra116.
- Walker TM, Ip CL, Harrell RH, Evans JT, Kapatai G, Dedicoat MJ, Eyre DW, Wilson DJ, Hawkey PM, Crook DW, *et al*. Whole-genome sequencing to delineate *Mycobacterium tuberculosis* outbreaks: a retrospective observational study. *Lancet Infect Dis* 2013;13:137–146.

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Interstitial Lung Disease Caused by STING-associated Vasculopathy with Onset in Infancy

To the Editor:

A full-term, male patient was born in the United Kingdom to parents of Hazars ethnic background, weighing 3.5 kg (50th percentile) and regaining his birth weight by Day 12. There was no significant family history, and he had two healthy siblings. He presented to hospital at 5 weeks of age with fever, respiratory distress, tachypnea, and a C-reactive protein level of 59 mg/L (reference range, <5 mg/L). During the next 6 months, he had a persistent cough and three further admissions. Chest radiographs showed persistent perihilar changes; he received multiple courses of

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antibiotics, although tests for bacterial and viral infections were consistently negative.

He was referred for specialist assessment at 7 months of age with ongoing respiratory symptoms, intermittent fever, and failure to thrive (weight, 5.59 kg; 0.4th percentile). He was tachypneic with moderate respiratory distress but had normal oxygen saturations in room air. He had clusters of raised maculopapular, erythematous lesions on his neck, back, arm, and legs first noted at 5 weeks of age (Figure 1A). Some of the features of this case have been previously reported in the form of an abstract (1).

Initial investigations found a C-reactive protein level of 10 mg/L, microcytic anemia with mean cell volume of 66.5 fl (reference range, 68–84 fl), and hemoglobin 100 g/L (reference range, 111–141 g/L). White blood cell count, renal function, liver function, thyroid function, and sweat chloride were normal. Chest radiograph showed increased interstitial and peribronchial markings throughout both lungs. Chest computed tomography showed features of interstitial lung disease (ILD; Figure 1B).

Immunological testing showed raised IgG (13.8 g/L; reference range, 3.0–9.0 g/L) and IgA (1.2 g/L; reference range, 0.2–0.7 g/L) levels, abnormal lymphocyte proliferation, highly positive antinuclear antibody screen with positive SS-A(Ro), and raised plasma viscosity (1.77 mPa · s; reference range, 1.50–1.72 mPa · s). Extensive investigations for bacteria, viruses, and fungi were performed on peripheral blood, skin biopsy, and bronchoalveolar lavage samples; all were negative.

A lung biopsy showed active inflammation with type 2 pneumocyte hyperplasia: epithelial hyperplasia of the bronchioles and surrounding lymphocytic infiltrate (Figure 1C). Ultrastructure examination with electron microscopy showed endothelial

tuboreticular inclusions, which are suggestive of excess type 1 IFN, as a result of either exogenous treatment or endogenous overproduction (Figure 1D).

Given the constellation of ILD, systemic inflammation, and persistent rash dating from 5 weeks of age, a diagnosis of STING-associated vasculopathy with onset in infancy (SAVI) was considered. Genetic analysis by Sanger sequencing confirmed a heterozygous somatic mutation (c. 463 G>A, p.V155M) in exon 5 of *TMEM173* (NM_198282.3), the gene encoding stimulator of interferon genes (STING). Genetic testing of the parents showed the mutation had occurred *de novo*. Whole-blood gene expression studies demonstrated a strong IFN signature, with IFN scores similar to those of other patients with SAVI (Figure 2).

Treatment with 3 days of intravenous methylprednisolone (10 mg/kg) yielded no clinical improvement. The patient gained weight with supplemental nasogastric feeding but had persistent tachypnea, and he subsequently became hypoxemic, requiring low-flow oxygen therapy. He then commenced monthly intravenous immunoglobulin at 2 g/kg; after 4 months of treatment, there has been some clinical improvement, with reduced tachypnea and a stable oxygen requirement.

The clinical syndrome of SAVI is characterized by early-onset (<8 wk of age) cutaneous vasculitis, fevers, ILD, and systemic inflammation. On laboratory testing, common features include positive autoantibodies (particularly a persistently raised antinuclear antibody) and raised IgG and IgA (2–5), all of which are features observed in our patient. Our patient developed a more central rather than peripheral (ears, nose, digits) rash exacerbated by cold exposure more commonly seen in patients with SAVI (6).

Figure 1. (A) Raised, papular, erythematous rash on the trunk. (B) Chest computed tomography scan showing widespread, slightly nodular interstitial opacification in both lungs. There are a number of small (<1 cm) peripheral cysts within the right lower and middle lobes. (C) Light microscopy microphotograph of lung biopsy (low magnification, hematoxylin and eosin stain), showing solid area composed of mixed infiltrate (left arrow) and chronic inflammation (right arrow). (D) Electron microscopy photograph of lung endothelium, showing tuboreticular inclusions (arrow).

Figure 2. Gene expression of selected IFN response genes was determined by Nanostring (NanoString Technologies, Seattle, WA), and an IFN score was calculated for healthy controls, patients with varying autoinflammatory conditions, and our patient (patient O6226). Standardized IFN score (y-axis) is the sum of six Nanostring counts that were standardized by subtracting the mean of healthy controls and dividing by the SD of the healthy controls. IFN response genes assessed in this study were *IFI27*, *IFI44*, *IFI44L*, *ISG15*, *RSAD2*, and *USP18*. Mean and SD of the IFN score are depicted in parentheses for each group of individuals. CANDLE = chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature syndrome (mediated by excess IFN); NOMID = neonatal onset multisystem inflammatory disease (mediated by excess IL-1 β); SAVI = STING-associated vasculopathy with onset in infancy (mediated by excess IFN).

Of the previously described cases, 11 of 12 patients had clinically apparent lung disease (3, 4, 6, 7). Importantly, three of these patients have died in adolescence from pulmonary complications (3, 7). Lung toxicity from exogenous type 1 IFN treatment has been reported in patients with multiple sclerosis (8, 9) and chronic hepatitis C virus infection (10). Despite the documented link between chronic type 1 IFN exposure and lung pathology, it is perplexing that SAVI is currently the only known type 1 interferonopathy in which lung involvement is a common and major clinical feature.

Autosomal-dominant, gain-of-function mutations in the *TMEM173* gene underlie the pathogenesis of SAVI. *TMEM173* encodes STING protein, an adaptor molecule linking sensing of foreign (viral and bacterial) DNA to the production of type 1 IFNs as part of the innate immune response. These gain-of-function mutations lead to constitutive activation of STING and upregulated type 1 IFN production. STING is expressed in alveolar macrophages, bronchial epithelium, and alveolar type 2 pneumocytes (3), which presumably explains the specific lung pathology seen in SAVI.

In addition, *in vitro* studies show that STING also acts directly on endothelial cells, causing inflammation and initiating the coagulation cascade. Thus, these *TMEM173* mutations are postulated to mediate chronic vessel endothelium inflammation, leading to the vasculitic rash and vasoocclusive processes seen in SAVI (3).

The predominance of early and significant respiratory symptoms, lack of characteristic peripheral rash in this patient, and variable phenotype described in family members

with the same, inherited *TMEM173* mutation (4) illustrates the variable phenotype of patients with SAVI; further research into the genotype–phenotype correlation and prognosis is warranted.

Treatment options in SAVI remain limited. Liu and colleagues reported a lack of response to glucocorticoids, disease-modifying antirheumatic drugs, and biological therapies (3). Isolated improvement in ILD has been documented in one patient after pulsed methylprednisolone and mycophenolate mofetil (6). Given the pathogenesis in SAVI, there is potential benefit from Janus kinase inhibitors to block type 1 IFN signaling, despite constitutively activated STING. The Janus kinase inhibitor baricitinib has shown benefit in adult patients with a range of inflammatory conditions, but there are limited pediatric data. An approved clinical protocol to assess the therapeutic benefit of baricitinib in patients with either SAVI or chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperatures (CANDLE) interferonopathy is ongoing and has recently published encouraging results in patients with CANDLE (ClinicalTrials.gov number NCT01724580) (11).

To conclude, a diagnosis of SAVI has significant implications for patients and families, not only from the high mortality risk but also from the significant morbidity. The respiratory component of the disease may predominate, and pulmonary complications have been the cause of death in previous reports. In the context of early age of onset, ILD, failure to thrive, fevers, and rash, we urge respiratory pediatricians to consider SAVI as a differential diagnosis and to request testing for *TMEM173* mutations and IFN gene signature to confirm the diagnosis. ■

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References

1. Pellowe EJ, Clarke SLN, Hilliard TN, Ramanana AV. Interstitial lung disease caused by STING-associated vasculopathy with onset in infancy (SAVI) [abstract]. *Thorax* 2015;70:A1-A254.
2. de Jesus AA, Canna SW, Liu Y, Goldbach-Mansky R. Molecular mechanisms in genetically defined autoinflammatory diseases: disorders of amplified danger signaling. *Annu Rev Immunol* 2015;33:823-874.
3. Liu Y, Jesus AA, Marrero B, Yang D, Ramsey SE, Montealegre Sanchez GA, Tenbrock K, Wittkowski H, Jones OY, Kuehn HS, et al. Activated STING in a vascular and pulmonary syndrome. *N Engl J Med* 2014;371:507-518.
4. Jeremiah N, Neven B, Gentili M, Callebaut I, Maschalidi S, Stolzenberg MC, Goudin N, Frémond ML, Nitschke P, Molina TJ, et al. Inherited STING-activating mutation underlies a familial inflammatory syndrome with lupus-like manifestations. *J Clin Invest* 2014;124:5516-5520.
5. Crow YJ, Manel N. Aicardi-Goutières syndrome and the type I interferonopathies. *Nat Rev Immunol* 2015;15:429-440.
6. Munoz J, Rodière M, Jeremiah N, Rieux-Laucat F, Oojageer A, Rice GI, Rozenberg F, Crow YJ, Bessis D. Stimulator of interferon genes-associated vasculopathy with onset in infancy: a mimic of childhood granulomatosis with polyangiitis. *JAMA Dermatol* 2015;151:872-877.
7. Omoyinmi E, Melo Gomes S, Nanthapisal S, Woo P, Standing A, Eleftheriou D, Klein N, Brogan PA. Stimulator of interferon genes-associated vasculitis of infancy. *Arthritis Rheumatol* 2015;67:808.
8. Petousi N, Thomas EC. Interferon- β -induced pulmonary sarcoidosis in a 30-year-old woman treated for multiple sclerosis: a case report. *J Med Case Reports* 2012;6:344.
9. Chakravarty SD, Harris ME, Schreiner AM, Crow MK. Sarcoidosis triggered by interferon-Beta treatment of multiple sclerosis: a case report and focused literature review. *Semin Arthritis Rheum* 2012;42:206-212.
10. Slavenburg S, Heijdra YF, Drenth JP. Pneumonitis as a consequence of (peg)interferon-ribavirin combination therapy for hepatitis C: a review of the literature. *Dig Dis Sci* 2010;55:579-585.
11. Montealegre G, Reinhart A, Brogan P, Berkun Y, Zlotogorski A, Brown D, Chira P, Gao L, Dare J, Schalm S, et al. Preliminary response to Janus kinase inhibition with baricitinib in chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperatures (CANDLE). *Pediatr Rheumatol Online J* 2015;13:O31.

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Short Stature and Access to Lung Transplantation: Reducing Disparities by Changing to a Lung Size-based Allocation Mechanism

To the Editor:

We read with great interest the article by Sell and colleagues on short stature and access to lung transplantation in the United States (1). Two important disparities in lung transplantation were identified: (1) short stature was associated with a lower rate of lung transplantation and higher rates of death and respiratory failure while awaiting transplantation, and (2) female patients were being disproportionately affected by this disparity. The authors proposed allocation policy adjustments, including waitlist prioritization of lung transplant candidates with a short stature.

We would like to expand the discussion on allocation policy. In the current organ allocation system, height is used to determine a limited range of donor lung size considered acceptable for a given lung transplant candidate (2-4). Although height is an important predictor of lung size, it fails to incorporate lung size difference associated with sex, age, and race (2-5). Independent of other measures, a man's lung is approximately 20% larger than a woman's (2-5). Similarly, differences in lung size between individuals of the same height but different races can reach 10 to 20% of organ size (2). Height clearly provides an imperfect estimate of lung size.

Lung size can be better estimated using the predicted total lung capacity (pTLC). The pTLC is a lung size measure derived from regression equations on the basis of height, sex, age, and race. In a series of studies, we have shown that the donor-to-recipient pTLC ratio, as a refined estimate of organ size, performs better than current sizing practice using height alone for the prediction of outcomes after transplantation (3-6). Measuring lung size matching via the donor-to-recipient pTLC ratio largely explained the outcome differences observed with sex-mismatched transplantation (2-5). It is likely that using pTLC ratios as a basis for selection of acceptable donors might significantly improve the allocation of donor lungs, especially for male recipients of short stature (2-5).

A second issue is that there has been a preference to "undersize" donor lungs, especially for recipients with fibrotic diseases. Our studies (adjusted for all known risk factors for post-lung transplant mortality available in Scientific Registry of Transplant Recipients datasets, including transplant indication, center, and center volumes) showed that allografts undersized by pTLC ratio estimates were associated with worse outcomes, whereas oversized allografts were associated with improved survival (4). The lowest risk of mortality was associated with a donor-to-recipient pTLC ratio of 1.3, and a ratio of 1.5 had a similar relative risk for mortality as a pTLC ratio of 1.0 (4). Furthermore, we showed that within transplants for idiopathic pulmonary arterial hypertension, a condition wherein size differences appear randomly distributed, the pTLC ratio was a strong independent predictor of survival (5). In that study, the median survival was 831 days longer in the oversized cohort (mean pTLC ratio, 1.24) (5). We have shown that oversized allografts are associated with superior allograft function (6). Adjusting upward acceptable donor lung size (as assessed by pTLC ratio) should further increase the number of donor organs acceptable for transplantation into short-statured individuals.