BAT2021_Hidradenitis suppurativa: from clinic to bench and back

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ABSTRACT

Hidradenitis suppurativa (HS) is a chronic inflammatory disease presenting with nodules, abscesses, and fistulas on the apocrine gland-bearing skin. HS may be classified as sporadic, familial or syndromic (PASH, PAPASH, PASH/SAPHO overlapping), the latter one being rare and characterized by a constellation of conditions regarded as autoinflammatory in their origin.
BAT2021 aims to bring together medical, genetic, experimental and lifestyle data to create holistic health records (HHR), which will allow us to build a tailored approach of each patient.
The inclusion criteria for patient enrollment are the compliance to the diagnostic criteria for HS; patient's demographics, clinical signs, anatomic phenotype classification, lifestyle habits, severity classification and treatment (former and current) are documented.
DNA/RNA obtained from biological samples (predominantly saliva and skin biopsies) of HS patients will be analysed by whole exome sequencing, whole genome genotyping SNPs arrays and transcriptomics. Clinical and molecular data will be stored into a special platform developed for the purpose of the project and will be analysed using advanced algorithms of artificial intelligence to propose a novel stratification method that clinicians can use in daily clinical practice.

KEYWORDS

Hidradenitis suppurativa, clinical practice, research workflow, whole-exome sequencing, whole genome genotyping SNPs arrays, transcriptomic, stratification, genotype-phenotype correlation, therapeutic outcomes

1 Clinical background

Hidradenitis suppurativa (HS), also known as acne inversa, is a chronic, inflammatory, recurrent, debilitating skin disease (of the terminal hair follicle), clinically characterized by inflammatory nodules that progress into abscesses and draining tunnels with foul smelling. Three main clinical HS phenotypes have been proposed, namely the classic or axillary-mammary, follicular and gluteal ones [1]. More recently, Van der Zee et al. proposed six different phenotypes, including the regular, frictional furuncle, scarring folliculitis, conglobata, syndromic and ectopic types [2]. Additional clinical phenotypes and cluster classifications have also been reported [3-5], but a definitive consensus has not been reached and any of these classifications addresses a prediction of therapeutic outcome. IHS4 (International Hidradenitis Suppurativa Severity Score System) is a validated tool for the severity assessment of HS and is arrived at by the number of nodules (multiplied by 1) plus the number of abscesses (multiplied by 2) plus the number of draining tunnels (multiplied by 4). A total score of 3 or less signifies mild, 4-10 means moderate and 11 or higher correspond to severe disease [6].
HS has a profound impact on patients and their family life, leading to a high extent of emotional and physical distress, with social embarrassment, isolation, and depression [7]. With a prevalence in Europe varying between 0.3% and 1% [8], and a diagnosis often underestimated and usually delayed for 7.2 ± 8.7 years [9], HS is not a rare disease.
HS is associated with several other disorders: i) autoimmune or inflammatory comorbidities, particularly inflammatory bowel diseases, ii) rheumatologic diseases, such as seronegative spondyloarthropathies and Adamantiades–Behçet disease spondylarthritis and iii) malignancies, where the most severe complication is the development of squamous cell carcinoma in areas of chronically diseased HS skin. Other comorbidities associated with HS include obesity, dyslipidemia, diabetes mellitus, metabolic syndrome, hypertension, cardiovascular disease, secondary amyloidosis, lymphedema, polycystic ovary syndrome and sexual dysfunction. Finally, HS is also associated with mental comorbidity and psychosocial impairments [10]. HS is usually a sporadic disease but may more rarely occur as a familial disorder [11]. In a minority of patients, HS can present in combination with other diseases as a complex clinical syndrome. The main autoinflammatory syndromes characterized by the presence of HS are pyoderma gangrenosum (PG), acne and suppurative hidradenitis (PASH), pyogenic arthritis, PG, acne and suppurative hidradenitis (PAPASH), psoriatic arthritis, PG, acne and suppurative hidradenitis (PsAPASH), pustular psoriasis, arthritis, PG, synovitis, acne and suppurative hidradenitis (PsAPSASH) and PG, acne, suppurative hidradenitis, and ankylosing spondylitis (PASS) [12]. However, HS can also occur in the context of complex syndromes such as Familial Mediterranean Fever (FMF), synovitis, acne, pustulosis, hyperostosis and osteitis (SAPHO), follicular occlusion syndrome, Down syndrome, Keratitis-ichthyosis-deafness (KID) syndrome, Dowling-Degos disease and Bazex-Dupré-Christol syndrome [13].
Risk factors such as smoking, obesity and other lifestyle triggers have been linked to HS onset, while genetic factors are considered to play a crucial role in HS etiopathogenesis [14]. 30% of HS patients report a family history of HS; mutations in γ-secretase genes (*NCSTN*, *PSENEN* and *PSEN1*) have been identified as the most common genetic changes involved in HS familial cases and these variants lead to an impairment of Notch signaling. Notch signaling pathway dysregulation results in an alteration in the proliferation and differentiation of keratinocytes leading to disruption of the normal hair follicle cycle and the formation of follicular cysts, typical for HS [15]. Our group recently hypothesized HS as a member of neutrophilic dermatoses based on the elevated concentration of

the cytokines IL-1β and IL-17 in skin lesions [16]. Moreover, some of our collaborators deeply involved in this project have also identified patients with HS occurring in the context of autoinflammatory syndromes, showing that PASH and PAPASH patients bear genetic variants in genes coding for proteins of the inflammasomes such as *PSTPIP1*, *MEFV*, *NOD2* and *NLRP3* [17]. Moreover, the up regulation of pro-inflammatory cytokines/chemokines in both lesional skin and serum are involved in the multifactorial HS pathogenesis [18]. With several new gene mutations coming into play, such as those involved in the keratinization pathways [19], on the background of a dysregulated innate immune response to commensal microbes and alterations in the skin microbiome as well, HS can be regarded as a multifactorial, polygenic autoinflammatory disease [18].

Medical treatments in HS are aimed at reducing incidence and flares thus improving HS patients' quality of life. Mild cases are usually treated by topical antibiotics having anti-inflammatory properties. Widespread disease is treated by systemic antibiotics and most severe cases by biologics such as adalimumab (anti-TNFα), currently the only biologic approved by the United States Food and Drug Administration [20] and by European Medicines Agency for treatment of HS [20,21].

Surgical resection of irreversibly damaged skin is often required, but often leads to functional impairments [20]. Different clinical trials for biologics targeting IL-17, IL-1 (alpha and beta), IL-36 and Janus kinase (JAK) 1 signaling response are currently ongoing, but simple outcome measures or novel biological models are demands to measure the efficacy of treatments [22].

2 Patient's enrollment and biological samples collection

Acting as one of the clinical partners of the project, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico of Milan has a large outpatient clinic with specialization in HS. The inclusion criteria for patient enrollment are the compliance to the diagnostic criteria for HS [23]. Patient's demographics, clinical signs, anatomic phenotype classification, lifestyle habits, severity classification and treatments (former and current) are documented. For the data documentation, the REDCap platform is used.

The study population include approximately 300 patients with moderate-to-severe HS, of which most are sporadic. 6% of patients have a HS positive family history and 14 patients present a HS syndromic form (4 PASH patients, 3 PAPASH, 5 PASH/SAPHO overlapping, 1 SAPHO and 1 patient with PASS).

Before biological samples collection, all patients and their relatives provide written informed consent for genomic analysis (protocol no. 487_2020) and receive pre-test genetic counselling in accordance with guidelines; indeed, the occurrence of the same condition among family members is a key factor to consider. Pedigree analysis of the families with more than one member affected is very useful for determining the patterns of disease inheritance.

All biological samples are collected, stored, and used in agreement with the ethical and research guidelines set.

Currently, we have collected saliva from 200 HS patients through Oragene DNA collection Kit (for human DNA) that allows for a high-quality human DNA to assess biomarkers and genetic variants associated to HS, its severity and response to biologic therapy.

In collaboration with IRCCS Burlo Garofolo of Trieste, we have analyzed through Whole Exome Sequencing, 12 syndromic patients (PASH, PAPASH, PASH/SAPHO overlapping) and in the first report, we have demonstrated genetic variants involving genes regulating the keratinization process and vitamin D metabolism, suggesting that a dysregulation of these two pathways may contribute to the HS pathogenesis. Vitamin D has been predicted as able to regulate skin homeostasis by controlling proliferation and differentiation of hair follicle and the low levels of vitamin D observed in all studied patients support the idea that vitamin D insufficiency could be involved in PASH and PAPASH pathogenesis.

We have also recruited 9 familial cases of HS, two of which in collaboration with IRCCS Burlo Garofolo of Trieste and the Italian Association of HS patients, respectively. Genetic analyses of HS familial cases and their family members are ongoing.

Our group has collected HS skin biopsies from lesional, perilesional and unaffected tissue (approximately 2 cm from the lesional skin) from the same anatomical region. Important is i) to take biopsies from different kind of HS lesions, including abscesses, plaques and fistulae (in the same patient, if it is possible); ii) smaller lesions (up to 1 cm in diameter) such as cysts and inflammatory and non-inflammatory nodules , should be completely excised while a deep biopsy (extending to subcutaneous tissue) should be made from abscesses and fistulae and iii) typical sites, such as axillary or inguinal folds as well as anogenital area should be chosen for taking biopsy but having samples also from atypical sites, i.e. dorsum or cervical region as well as foruncles on different areas of the body, could be of interest.

Skin samples has been subdivided into two parts, one of which for conventional histology (formalin-fixed, paraffin-embedded) and the other one frozen for additional studies (immunohistochemistry, protein array, real - time PCR). An additional skin samples is taken and stored in Rna ladder for transcriptomic analyses.

For functional and validation studies, we have performed hair follicle pick up according to the following procedure: a firm pull motion with forceps must be performed at the base of the hair. Only plucked hair in the anagen phase (minimum of five from each subject) contain enough keratinocytes for a successful culture initiation. The hair has been plucked from the occipital and temporal scalp regions but facial hair types like beard, eyebrow, or hair from the nose can be used. The hair shaft has been cutted slightly behind the follicle with sterile scissors resulting in an approximate 5 mm long piece consisting mainly of the follicle. The plucked hairs were stored in a tube filled with 5 mL Defined keratinocytes-SFM medium (DKSFM; Gibco – Thermo Fisher Scientific, Switzerland) at room temperature [24].

3 Conclusions

The comparison of the results obtained from DNA/RNA sequencing between patients and controls will highlight possible causative genes and signalling pathways. The possible detection of genotype-phenotype correlations will allow a more exhaustive and precise clinical patient stratification which, in addition to the existing pharmacogenetic data banks, will help the development of new effective drugs and a future individualized treatment of HS patients.

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REFERENCES

[1] Canoui-Poitrine F, Le Thuaut A, Revuz JE, Viallette C, Gabison G, Poli F, et al. (2013). Identification of three hidradenitis suppurativa phenotypes: latent class analysis of a cross-sectional study. J Invest Dermatol, 133(6):1506-11. doi: 10.1038/jid.2012.472

[2] Van der Zee HH, Jemec GB. (2015). New insights into the diagnosis of hidradenitis suppurativa: Clinical presentations and phenotypes. JAMA, 73: S23-6. doi: 10.1016/j.jaad.2015.07.047

[3] Martorell A, Jfri A, Koster SBL, Gomez-Palencia P, Solera M, Alfaro-Rubio A, et al. (2020). Defining hidradenitis suppurativa phenotypes based on the elementary lesion pattern: results of a prospective study. J Eur Acad Dermatol Venereol, 34(6):1309-1318. doi: 10.1111/jdv.16183

[4] Naasan H, Affleck A. (2015). Atypical hidradenitis suppurativa. Clin Exp Dermatol, 40:891-3. doi: 10.1111/ced.12655

[5] Cazzaniga S, Pezzolo E, Bettoli V, Abeni D, Marzano AV, Patrizi A, et al. (2021). Characterization of Hidradenitis Suppurativa Phenotypes: A Multidimensional Latent Class Analysis of the National Italian Registry IRHIS. J Invest Dermatol, 141(5):1236-1242.e1. doi: 10.1016/j.jid.2020.08.032

[6] Zouboulis CC, Tzellos T, Kyrgidis A, Jemec GBE, Bechara FG, Giamarellos-Bourboulis EJ, et al. (2017). Development and validation of the International Hidradenitis Suppurativa Severity Score System (IHS4), a novel dynamic scoring system to assess HS severity. Br J Dermatol, 177(5):1401-1409. doi: 10.1111/bjd.15748.

[7] Matusiak Ł. (2020). Profound consequences of hidradenitis suppurativa: a review. Br J Dermatol, 183(6): e171-e177. doi: 10.1111/bjd.16603

[8] Ingram JR. (2020) The epidemiology of hidradenitis suppurativa. Br J Dermatol, 183(6):990-998. doi: 10.1111/bjd.19435

[9] Saunte DM, Boer J, Stratigos A, Szepietowski JC, Hamzavi I, Kim KH, et al. (2015). Diagnostic delay in hidradenitis suppurativa is a global problem. Br J Dermatol, 173(6):1546-9. doi: 10.1111/bjd.14038

[10] Preda-Naumescu A, Ahmed HN, Mayo TT, Yusuf N. (2021) Hidradenitis suppurativa: pathogenesis, clinical presentation, epidemiology, and comorbid associations. Int J Dermatol. doi: 10.1111/ijd.15579. Epub ahead of print.

[11] Zouboulis CC, Benhadou F, Byrd AS, Chandran NS, Giamarellos-Bourboulis EJ, Fabbrocini G, et al. (2020). What causes hidradenitis suppurativa?-15 years after. Exp Dermatol, 29(12):1154-1170. doi: 10.1111/exd.14214.

[12] Genovese G, Moltrasio C, Garcovich S, Marzano AV. (2020) PAPA spectrum disorders. G Ital Dermatol Venereol, 155(5):542-550. doi: 10.23736/S0392-0488.20.06629-8.

[13] Garcovich S, Genovese G, Moltrasio C, Malvaso D, Marzano AV. PASH, PAPASH, PsAPASH, and PASS: The autoinflammatory syndromes of hidradenitis suppurativa. (2021). Clin Dermatol, 39(2):240-247. doi: 10.1016/j.clindermatol.2020.10.016.

[14] Sabat R, Jemec GBE, Matusiak Ł, Kimball AB, Prens E, Wolk K. Hidradenitis suppurativa. (2021). Nat Rev Dis Primers, 6(1):18. doi: 10.1038/s41572-020-0149-1

[15] Wang Z, Yan Y, Wang B. γ-Secretase Genetics of Hidradenitis Suppurativa: A Systematic Literature Review. (2020). Dermatology, 1-7. doi: 10.1159/000512455. Epub ahead of print.

[16] Marzano AV, Ortega-Loayza AG, Heath M, Morse D, Genovese G, Cugno M. Mechanisms of Inflammation in Neutrophil-Mediated Skin Diseases. (2019). Front Immunol, 10:1059. doi: 10.3389/fimmu.2019.01059

[17] Marzano AV, Damiani G, Ceccherini I, Berti E, Gattorno M, Cugno M. (2017). Autoinflammation in pyoderma gangrenosum and its syndromic form (pyoderma gangrenosum, acne and suppurative hidradenitis). Br J Dermatol, 176(6):1588-1598. doi: 10.1111/bjd.15226

[18] Marzano AV. (2016). Hidradenitis suppurativa, neutrophilic dermatoses and autoinflammation: what's the link?. Br J Dermatol, 174(3):482-3. doi: 10.1111/bjd.14364

[19] Brandao L, Moura R, Tricarico PM, Gratton R, Genovese G, Moltrasio C, et al. (2020). Altered keratinization and vitamin D metabolism may be key pathogenetic pathways in syndromic hidradenitis suppurativa: a novel whole exome sequencing approach. J Dermatol Sci, 99(1):17-22. doi: 10.1016/j.jdermsci.2020.05.004

[20] Folkes AS, Hawatmeh FZ, Wong A, Kerdel FA. Emerging drugs for the treatment of hidradenitis suppurativa. (2020). Expert Opin Emerg Drugs, 25(3):201-211. doi: 10.1080/14728214.2020.1787984.

[21] Marzano AV, Genovese G, Casazza G, Moltrasio C, Dapavo P, Micali G, et al. (2021). Evidence for a 'window of opportunity' in hidradenitis suppurativa treated with adalimumab: a retrospective, real-life multicentre cohort study. Br J Dermatol, 184(1):133-140. doi: 10.1111/bjd.18983.

[22] Zouboulis CC, Frew JW, Giamarellos-Bourboulis EJ, Jemec GBE, Del Marmol V, Marzano AV, et al. (2021). Target molecules for future hidradenitis suppurativa treatment. Exp Dermatol, 30 Suppl 1:8-17. doi: 10.1111/exd.14338. PMID: 34085329.

[23] Zouboulis CC et al. (2015). Hidradenitis suppurativa/acne inversa: Criteria for diagnosis, severity assessment, classification and disease evaluation. Dermatology, 231:184-90. doi: 10.1159/000431175

[24] Re S, Dogan AA, Ben-Shachar D, Berger G, Werling AM, Walitza S, et al. (2018). Improved Generation of Induced Pluripotent Stem Cells From Hair Derived Keratinocytes - A Tool to Study Neurodevelopmental Disorders as ADHD. Front Cell Neurosci, 12:321. doi: 10.3389/fncel.2018.00321.