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ORIGINAL ARTICLE

A retrospective analysis of diagnostic testing in a large North American cohort of patients with epidermolysis bullosa

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Brooklyn and New York, New York; Cincinnati, Ohio; Aurora, Colorado; Stanford and San Diego, California; Toronto, Ontario, Canada; Worcester, Massachusetts; Minneapolis, Minnesota; Chicago, Illinois; Montreal, Quebec, Canada; Philadelphia, Pennsylvania; Austin, and San Antonio, Texas; Phoenix, Arizona; Miami, Florida; Detroit, Michigan; and St. Louis, Missouri

Background: Accurate diagnosis of epidermolysis bullosa (EB) has significant implications for prognosis, management, and genetic counseling.

Objective: To describe diagnostic testing patterns and assess diagnostic concordance of transmission electron microscopy (TEM), immunofluorescence mapping (IFM), and genetic analysis for EB.

Methods: A retrospective cohort included patients enrolled in the Epidermolysis Bullosa Clinical Characterization and Outcomes Database from January 1, 2004, to July 8, 2019. Tests concluding the same EB type (EB simplex, junctional EB, dominant dystrophic EB, and recessive dystrophic EB) were considered concordant; those concluding different EB types were considered discordant; and those with nonspecific/nondefinitive results were equivocal.

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Results: A total of 970 diagnostic tests were conducted from 1984 to 2018 in 771 patients. Genetic analyses were performed chronologically later than IFM or TEM ($P < .001$). The likelihood of undergoing genetic analysis was greater for junctional EB and recessive dystrophic EB, and the same for dominant dystrophic EB as compared with EB simplex. TEM results in 163 patients were equivocal (55%), concordant (42%), and discordant (3%). IFM results in 185 patients were equivocal (54%), concordant (42%), and discordant (4%).

Limitations: Retrospective design.

Conclusions: Diagnostic testing has shifted in favor of genetic analysis. TEM and IFM frequently offer equivocal findings when compared to the specificity afforded by genetic analysis. (J Am Acad Dermatol https://doi.org/10.1016/j.jaad.2021.09.065.)

Key words: diagnostic concordance; diagnostic testing; electron microscopy; epidermolysis bullosa; genetic analysis; genetics; immunofluorescence mapping; laboratory testing; next-generation sequencing.

INTRODUCTION

Epidermolysis bullosa (EB) is a heterogeneous group of mechanobullous disorders resulting from mutations in genes encoding structural proteins of the skin.[1,2] With thousands of known mutations in at least 21 structural genes, the clinical spectrum of disease ranges widely from mild, friction-induced blisters of the extremities to severe, congenital mucocutaneous fragility accompanied by extracutaneous complications and limited lifespan.[3-6]

There are 4 major EB types: EB simplex (EBS), junctional EB (JEB), dystrophic EB (DEB), and Kindler EB (KEB), with over 30 clinical subtypes.[3,7] Classification schemes have evolved[8,9] to incorporate biomolecular techniques, including transmission electron microscopy (TEM),[10] immunofluorescence mapping (IFM),[11] and genetic analysis in addition to clinical phenotype and inheritance patterns.[2,7,10,12-14] Because distinguishing the major types of EB on clinical features alone can be unreliable, especially in the neonatal period, rapid and accurate laboratory diagnosis is essential.

Establishing an accurate and timely diagnosis and subtype specification of EB has implications for prognosis, management, and counseling because different forms of EB have distinct clinical features and complications that evolve over time. However, in light of the rarity of EB, limited data are available on variation in real-world diagnostic testing utilization, especially in a large, longitudinal cohort. In particular, the timing and associated costs of diagnostic tests play intricate roles in resource utilization, which ultimately have downstream effects on patient outcomes. Recent guidelines[3] for implementing various laboratory diagnostic tests have weighed the utility of TEM, IFM, and genetic analysis, but there remains a need to establish generalizable data to inform EB diagnosis in practice.

CAPSULE SUMMARY

- Limited data are available on the real-world utilization and diagnostic concordance of laboratory testing for epidermolysis bullosa.
- Our study supports the use of genetic analysis for the diagnosis of epidermolysis bullosa in all cases. In addition, we suggest genetic analysis be considered over skin biopsy for neonates with skin fragility.

We sought to define the diagnostic testing patterns in a large cohort of EB patients and assess the diagnostic concordance of these tests in order to inform management recommendations. To do so, we utilized a large, contemporary database managed by the Epidermolysis Bullosa Clinical Research Consortium,[15] and tracked the utilization and diagnostic concordance of TEM, IFM, and genetic analysis over 4 decades.

METHODS

Data source and study population

The Epidermolysis Bullosa Clinical Characterization and Outcomes Database (EBCCOD) has been described previously.[15] It constitutes the clinical data collected contemporaneously and retrospectively from 20 sites in the United States, Canada, and Mexico. The data are housed and managed at the University of Colorado, Denver. Participation in the database is approved by the institutional review board at each participating institution. All patients give written informed consent/assent upon enrollment.

Patients from 18 participating sites who were enrolled in the EBCCOD between January 1, 2004,

Abbreviations used:

DDEB:	dominant dystrophic epidermolysis bullosa
EB:	epidermolysis bullosa
EBCCOD:	Epidermolysis Bullosa Clinical Characterization and Outcomes Database
IFM:	immunofluorescence mapping
IQR:	interquartile range
JEB:	junctional epidermolysis bullosa
KEB:	Kindler epidermolysis bullosa
NGS:	next-generation sequencing
RDEB:	recessive dystrophic epidermolysis bullosa
TEM:	transmission electron microscopy

and July 8, 2019, and who had data available from diagnostic testing were included. Patients were initially diagnosed with EB between 1952 and 2018. Given the mid-year enrollment cutoff, December 31, 2018, was chosen as the cutoff for diagnostic tests (4 tests were reported in 2019, all for patients who were initially diagnosed with EB prior to 2019).

Type of EB was categorized as EBS, JEB, dominant dystrophic epidermolysis bullosa (DDEB), and recessive dystrophic epidermolysis bullosa (RDEB). A fifth category of unknown/other (including DEB not otherwise specified and KEB) was included in the descriptive summary but omitted from statistical analysis due to the nonspecific diagnostic information and the low number of definitive KEB diagnoses ($n = 4$).

Diagnostic testing assessment

Patient data abstracted from the database included demographics, EB type, and chronology and results of TEM, IFM, and genetic analysis. Diagnostic concordance between TEM, IFM, and genetic analysis was evaluated with genetic analysis as the reference standard. Tests concluding the same EB type (eg, EBS, JEB, DDEB, RDEB) were considered to be concordant; those with different EB types were considered discordant; and those with nonspecific/nondefinitive results, were considered equivocal (eg, EB type could not be definitively concluded due to the absence of clefting on IFM or TEM; or IFM or TEM concluded DEB, but could not specify DDEB vs RDEB). Instances in which genetic analysis was inconclusive were reported separately.

Statistical analysis

Descriptive statistics were used to describe study participants and other outcomes of interest. Time-to-event analysis was performed using Cox regression analysis. A 2-sided P value of $<.05$ was considered statistically significant. Statistical analyses were performed using Excel (Microsoft Corp) and SPSS Statistics version 26 (IBM).

RESULTS

Patient characteristics

Of 854 patients identified in the EBCCOD during the study period, 771 (90%) had information available on the utilization of TEM, IFM, and/or genetic analysis (Supplementary Fig 1, available via Mendeley at https://doi.org/10.17632/s9wyv3g982.1). Our study cohort consisted of 319 (41%) RDEB, 213 (28%) EBS, 120 (16%) DDEB, 74 (10%) JEB, and 45 (6%) unknown/other patients (Table I). The yearly composition of the study cohort remained relatively stable over the study period. The proportion of EBS patients ranged from 18% to 26%; JEB, 2% to 10%; DDEB, 13% to 22%; and RDEB, 42% to 56%.

Diagnostic testing patterns and chronology

In total, 970 diagnostic tests were reported in the 34-year period from 1984 to 2018. Genetic analysis was the most frequently reported diagnostic test (464 of 760; 61%), followed by IFM (285 of 682; 42%) and TEM (221 of 663; 33%) (Table I). Median age at testing was greatest for genetic analysis (24.5 months) compared to TEM (1.8 months) and IFM (1.0 months). The rate of genetic testing was 67% (220 of 327) among those born between 2009 and 2019 and 69% (48 of 70) for those born between 2016 and 2019. Genetic mutations in *COL7A1*, *KRT14*, and *COL17A1* were most commonly reported in 279, 43, and 41 patients, respectively (Supplementary Table I, available via Mendeley at https://doi.org/10.17632/s9wyv3g982.1). JEB and RDEB patients were more likely to have received any of TEM, IFM, or genetic analysis, while EBS patients were less likely to have had testing ([JEB: OR 3.7; 95% CI, 1.6-8.8], [RDEB: OR 3.0; 95% CI, 2.0-4.5], and [EBS: OR, 0.3; 95% CI, 0.2-0.4]). No statistically significant relationship between having any diagnostic test and sex was identified ($P > .2$).

Genetic analysis was more frequently performed on patients ultimately diagnosed with JEB and RDEB versus EBS (Table I) ([JEB: OR, 2.1; 95% CI, 1.2-3.7], [RDEB: OR, 2.3; 95% CI, 1.7-3.2], and [EBS: OR, 0.4; 95%, 0.3-0.5]). Median age at testing was greatest for RDEB patients (48 months; interquartile range [IQR] 4-139 months, $P = .01$). By Cox regression analysis accounting for patient sex, the likelihood of undergoing testing was greater for JEB and RDEB, and the same for DDEB as compared to EBS (Table II).

Fig 1 depicts diagnostic test frequency over time. Genetic analyses were generally performed chronologically later than IFM or TEM (median [IQR] test date: April 2014 [May 2010 to March 2016] vs April

Table I. Patient characteristics and diagnostic testing utilization

Demographics	Total*	EB type*				*P* value†
		EBS	JEB	DDEB	RDEB	
Age at diagnosis, median (IQR), mo (*N* = 606)‡	1.0 (0-4.9)	2.0 (0-12)	1.0 (0-3.9)	1.0 (0-3.0)	0 (0-1.0)	<.001
Sex, *N* = 725§						.567
Male	369 (50.9)	114 (53.5)	36 (48.6)	55 (45.8)	164 (51.6)	
Female	356 (49.1)	99 (46.5)	38 (51.4)	65 (54.2)	154 (48.4)	
Race, *N* = 726						<.001
American Indian/Alaska Native	5 (0.7)	0 (0)	3 (4.1)	1 (0.8)	1 (0.3)	
Asian	46 (6.3)	6 (2.8)	5 (6.8)	6 (5)	29 (9.1)	
Native Hawaiian/Pacific Islander	3 (0.4)	1 (0.5)	0 (0)	0 (0)	2 (0.6)	
Black/African American	48 (6.6)	19 (8.9)	9 (12.2)	14 (11.7)	6 (1.9)	
White	474 (65.3)	154 (72.3)	34 (45.9)	79 (65.8)	207 (64.9)	
Middle Eastern/North African	67 (9.2)	8 (3.8)	15 (20.3)	7 (5.8)	37 (11.6)	
Unknown	83 (11.4)	25 (11.7)	8 (10.8)	13 (10.8)	37 (11.6)	
Diagnostic testing history						
Any, *N* = 602	442 (73.4)	92 (53.2)	53 (89.8)	73 (70.2)	224 (84.2)	<.001
Multiple, *N* = 602	243 (40.4)	42 (24.3)	33 (55.9)	36 (34.6)	132 (49.6)	<.001
Transmission electron Microscopy, *N* = 625	214 (34.2)	42 (23.5)	26 (43.3)	30 (28.6)	116 (41.3)	<.001
Age at testing, median (IQR), mo (*N* = 177) ‡	1.8 (0.3-47.5)	4.5 (1.0-19.2)	1.0 (0.2-35.1)	3.0 (1.0-40.9)	1.5 (0.1-53.7)	.6
Immunofluorescence Mapping, *N* = 644	274 (42.5)	56 (29.6)	40 (60.6)	39 (35.8)	139 (49.6)	<.001
Age at testing, median (IQR), mo *N* = 230†	1.0 (0.1-16.0)	3.0 (0.4-13.6)	1.0 (0.2-5.0)	1.4 (0.4-9.2)	1.0 (0.1-51.0)	.9
Genetic analysis, *N* = 715	445 (62.2)	91 (44)	56 (75.7)	70 (58.8)	228 (72.4)	<.001
Age at testing, median (IQR), mo (*N* = 390) ‡	24.5 (3.6-116.7)	18.9 (3.0-62.8)	13.5 (2.2-59.8)	18.1 (4.7-152.9)	48.0 (4.0-138.7)	.01

DDEB, Dominant dystrophic epidermolysis bullosa; *EB*, epidermolysis bullosa; *EBS*, epidermolysis bullosa simplex; *IQR*, interquartile range; *JEB*, junctional epidermolysis bullosa; *RDEB*, recessive dystrophic epidermolysis bullosa.

*Values presented as number (%) except where otherwise stated as median (IQR).

†*P* values were determined by chi-square or Kruskal-Wallis between EB types.

‡Prenatal diagnosis or diagnostic testing were excluded for these calculations.

§Total numbers of patients (N) presented in Table I are specific to the data available for each variable; for example, 725 patients had both a reported EB type and sex, while 715 had both a reported EB type and genetic analysis status (yes/no).

2011 [October 2006 to February 2014]) and December 2009 [March 2004 to September 2013], respectively; ($P < .001$), while TEM and IFM median test dates were not appreciably different from one another ($P = .1$). The rate of genetic testing per eligible patient per year surpassed TEM and IFM in 2008. Single-year genetic analysis test frequency was highest in 2016.

Diagnostic testing concordance

Diagnostic concordance between TEM and genetic analysis among 163 patients with results available showed TEM to be largely equivocal (55%) or concordant (42%) and rarely discordant (3%) with genetic analysis (Fig 2). Similarly, IFM results among 185 patients with results available were equivocal, concordant, or discordant in comparison to genetic analysis in 54%, 42%, and 4%, respectively. Supplementary Tables II and III (available via Mendeley at https://doi.org/10.17632/s9wyv3g982.1) show diagnostic concordance stratified by clinical subtype. Among the 212 patients with genetic analysis plus TEM and/or IFM results, there were 8 (4%) instances of genetic testing that yielded inconclusive results. Six of these resulted from limited testing of candidate genes, and in 2 patients, DDEB and RDEB were unable to be reliably distinguished.

The proportion of equivocal cases within each EB type was highest in DEB patients for both TEM (85% DDEB, 67% RDEB, 22% EBS, 20% JEB; $P < .001$) and IFM (86% DDEB, 63% RDEB, 28% EBS, 15% JEB; $P < .001$). Supplementary Table IV (available via Mendeley at https://doi.org/10.17632/s9wyv3g982.1) and Supplementary Fig 2 (available via Mendeley at https://doi.org/10.17632/s9wyv3g982.1) summarize the odds of diagnostic concordance by EB type.

Table II. Results of Cox regression analyses of time to test (age, years)

Variable	Hazard ratio	95% CI	*P* value
Any test			
JEB vs EBS	2.339	1.650-3.315	<.001
DDEB vs EBS	1.161	0.842-1.600	.363
RDEB vs EBS	1.649	1.281-2.122	<.001
Male sex (reference, female)	1.247	1.023-1.521	.029
Transmission electron microscopy			
JEB vs EBS	2.459	1.456-4.152	.001
DDEB vs EBS	1.112	0.662-1.868	.688
RDEB vs EBS	1.865	1.257-2.767	.002
Male sex (reference, female)	1.251	0.926-1.690	.144
Immunofluorescence mapping			
JEB vs EBS	2.853	1.836-4.433	<.001
DDEB vs EBS	1.281	0.821-1.998	.275
RDEB vs EBS	1.810	1.272-2.574	.001
Male sex (reference, female)	1.033	0.795-1.343	.806
Genetic analysis			
JEB vs EBS	2.162	1.530-3.055	<.001
DDEB vs EBS	1.053	0.758-1.462	.760
RDEB vs EBS	1.356	1.049-1.753	.020
Male sex (reference, female)	1.178	0.963-1.442	.111

DDEB, Dominant dystrophic epidermolysis bullosa; *EBS*, epidermolysis bullosa simplex; *JEB*, junctional epidermolysis bullosa; *RDEB*, recessive dystrophic epidermolysis bullosa.

DISCUSSION

We present data on a large cohort of patients with EB that depict real-world utilization of various EB diagnostic testing modalities over the last 4 decades. We observed that TEM was the predominant testing modality in the first half of the study period, 1984-2001, until IFM gained predominance 20 years after its introduction in 1981.[11,16] IFM has been found to be more sensitive (97% vs 71%) and specific (100% vs 81%) than TEM using genetic analysis as a reference standard,[17] and is less time-consuming and operator dependent that TEM.[3,18]

Ultimately, our results show that diagnostic testing shifted in favor of genetic analysis over IFM or TEM, with genetic analyses on average being performed more recently than IFM or TEM. Sanger sequencing for EB diagnosis was introduced in 1991[19,20] and was typically preceded by IFM and TEM due to sequencing's prolonged turnaround time and candidate gene preselection requirements.[3] This traditional paradigm is reflected in our data by the younger median age at TEM and IFM testing versus genetic analysis. Regardless, the rate of genetic analysis testing[20-28] surpassed TEM and IFM in our cohort in 2008.

We observed a peak in genetic analysis testing in 2016 after the introduction of whole-exome sequencing and next-generation sequencing (NGS) gene panels for the diagnosis of EB in 2015, which drastically improved cost and time efficiency.[29-33] Superiority of NGS over IFM has been suggested where a NGS multigene panel established the diagnosis in 90% of cases compared to 76% with IFM.[33] Moreover, the increasing rate of genetic analysis for participants born 2009 or later, and even greater rate for those born after the introduction of NGS panels for EB in 2016, emphasizes the trend toward genetic testing.

EB type emerged as a significant factor in the likelihood of undergoing diagnostic testing. The increased utilization of diagnostic tests among JEB and RDEB patients may reflect the increased acuity and morbidity associated with many forms of JEB and RDEB that would require increased contact with the medical system and tertiary EB centers, as well as precise subtype specification for the management and counseling of these patients.

We also compared diagnostic testing results between TEM, IFM, and genetic analysis, observing that while TEM and IFM could corroborate a diagnosis, they frequently offered equivocal findings when compared to the specificity afforded by genetic analysis. This was particularly true for forms of DEB, where DDEB and RDEB both share subepidermal cleavage planes (TEM and IFM), reduced anchoring fibrils (TEM), and reduced or absent collagen VII (IFM). IFM has been shown to have less sensitivity for the diagnosis of EBS and JEB compared to DEB.[34]

Of the 4 EB types used in our analysis, the odds of a concordant TEM test result was highest for EBS patients, while the odds of a concordant IFM test result was highest for JEB patients. Although our analytical framework did not account for the clinical context underscoring these diagnostic tests, our results suggest greater utility for TEM with EBS patients and IFM for JEB patients relative to other EB types.

The high success rate of genetic analysis we observed overall (96%) suggests that TEM and IFM, which require a skin biopsy, should be reserved for cases in which NGS or Sanger sequencing fail to establish a definitive diagnosis or provide adequate prognostic information. For example, in cases in which genetic testing fails to identify pathogenic variants in EB-associated genes or identifies variants of unknown significance, expression or functional studies may be required in addition to immunostaining

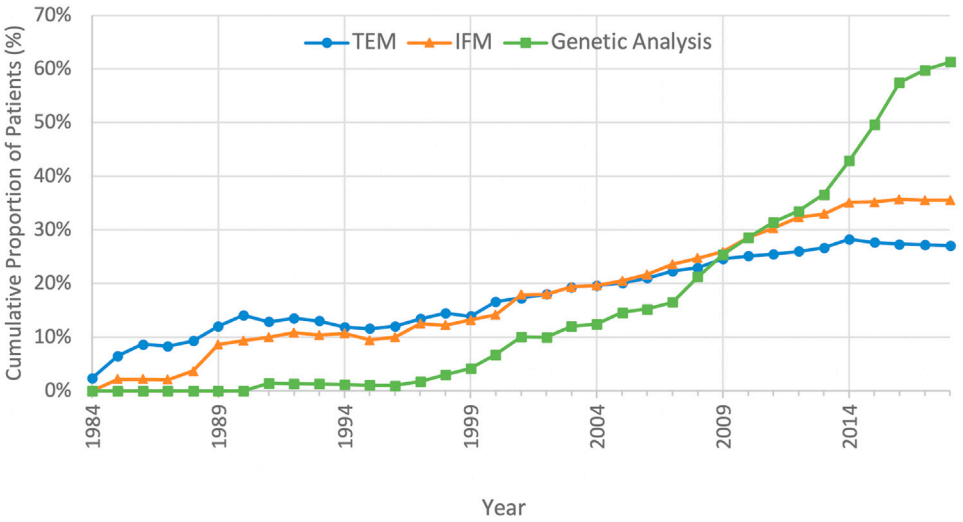

Fig 1. Timeline of relative diagnostic testing utilization among the EBCCOD: cumulative proportion of patients with diagnostic testing 1984-2018 normalized to the cumulative number of patients in the database. *EBCCOD*, Epidermolysis Bullosa Clinical Characterization and Outcomes Database; *IFM*, immunofluorescence mapping; *TEM*, transmission electron microscopy.

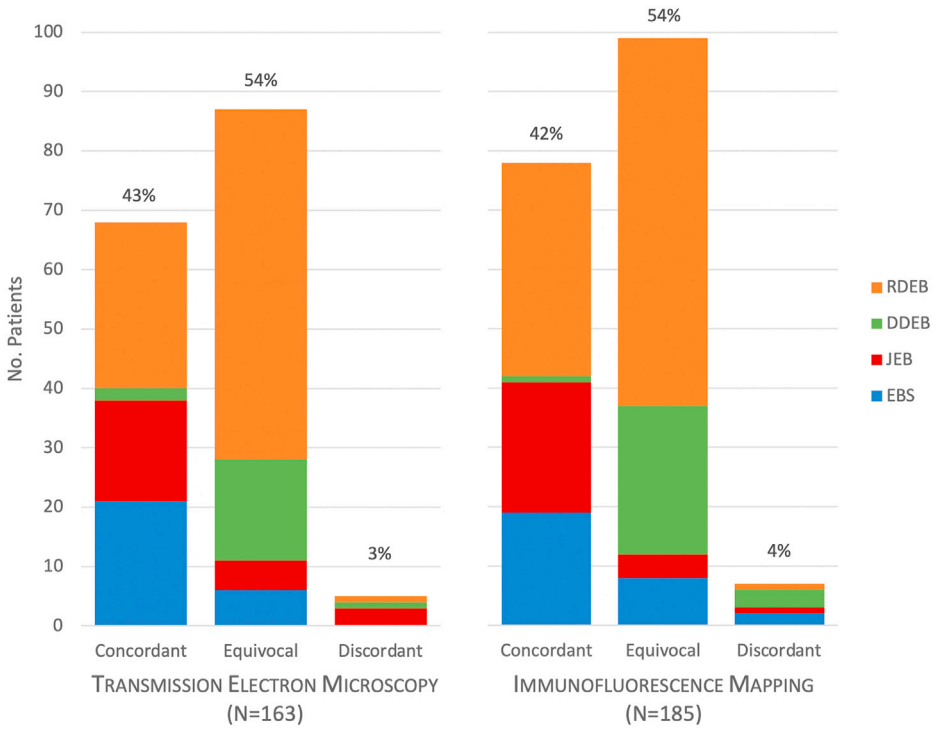

Fig 2. Diagnostic concordance of transmission electron microscopy or immunofluorescence mapping compared to genetic analysis stratified by EB type. *DDEB*, Dominant dystrophic epidermolysis bullosa; *EBS*, epidermolysis bullosa simplex; *JEB*, junctional epidermolysis bullosa; *RDEB*, recessive dystrophic epidermolysis bullosa.

pattern and intensity data provided by IFM.[3] Additionally, in instances where severe JEB is suspected in a neonate, tandem genetic testing and IFM would contribute to rapid diagnosis and tailored management.

While an in-depth analysis of the factors contributing to absence of a genetic diagnosis in 39% of our cohort is outside the scope of this study, likely contributors have historically included limited availability, prohibitive cost, and prolonged turnaround

time of genetic analysis. Care of EB in general has been shown to pose a high financial burden on patients and caregivers in the United States,[35] which lacks the centralized health system and broad insurance coverage for genetic testing that we imagine has facilitated the high rates of genetic testing reported in another registry.[36] The lag between observation of clinical signs and subsequent confirmatory testing, particularly genetic analysis, has the potential for significant ramifications for patient outcomes.[15]

Current guidelines recommend tandem genetic analysis and skin biopsy for IFM for neonates with skin fragility in order to inform neonatal management within hours to days as opposed to days to weeks using genetic analysis alone.[3] However, commercial diagnostic genetic analysis capabilities have since advanced following the publication of these guidelines. In fact, some US-based genetic testing services offer turnaround times as short as 1 week for whole-exome sequencing in emergent cases.[37-39] Moreover, in a survey of several commercial NGS panels for EB, self-pay costs have decreased by 13% to 51% to as low as $890 between 2018 and 2021.[40-43] We anticipate that as commercially available clinical genetic analysis capabilities continue to advance[44] and reimbursement for diagnostic testing equilibrates with consensus- and evidence-driven best practices, EB nosology and diagnostic algorithms will continue to be refined. Formal quantitative cost-analysis studies are needed to fully capture the value of various diagnostic tests, which remains difficult for a rare congenital disorder in a dynamic genomics landscape.

In summary, our results demonstrate increased utilization of genetic testing for EB and support recent guidelines that EB laboratory diagnosis should be performed and that genetic testing is recommended for the diagnosis of EB.[3] We concur that genetic analysis allows for the following: (1) precise diagnosis,[7,33,45] (2) prenatal testing and counseling, (3) preimplantation testing and counseling,[46-50] (4) prognostication, and (5) pathogenetic-directed therapy.[5,51]

While the EBCCOD is a large, multicenter, longitudinal, and contemporary database of North American EB patients, our dataset is biased toward patients who present to the participating specialized EB centers; eg, toward patients with severe enough disease to warrant ongoing management and access to specialized care, but against patients with rapidly lethal forms of EB. This would explain the relatively lower number of EBS patients in our cohort than expected. Furthermore, not all data points of interest were available for every enrolled participant in the database, which likely skews toward more recently enrolled participants. In addition, the lack of granularity in the genetic analysis data precluded comparisons of the various genetic analysis methods, including whether multiple stages of genetic analysis were required.

There was great variance in which laboratories were used for all testing formats, making comparison of results more difficult. As mentioned above, the diagnostic concordance schemes may have underestimated the utility of IFM and TEM for DEB because concordance with genetic analysis frequently required the differentiation of RDEB and DDEB. Finally, we cannot draw any direct conclusions about the accuracy of genetic analysis because it acted as the reference standard when diagnostic concordance was established with TEM and IFM.

CONCLUSIONS

Our analysis of diagnostic testing for EB in North America over the past 4 decades revealed a definitive shift toward genetic analysis over TEM and IFM that correlated with technological advances in the field. EB type emerged as a significant factor in the likelihood of receiving TEM, IFM, or genetic analysis, with JEB and RDEB most likely to have received diagnostic laboratory testing. TEM and IFM revealed equivocal diagnoses in comparison to genetic analysis in more than half of cases, emphasizing the specificity afforded by genetic analysis. As turnaround time and cost of genetic analysis continue to improve, we anticipate increased utilization of genetic analysis for precise diagnosis of EB with subtype specification for optimal prognostication and counseling.

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Conflicts of interest

Dr Bruckner serves as an investigator for Fibrocell, Phoenix Tissue Repair, PROQR/Wings, and Castle Creek and on an ad hoc advisory board for Castle Creek. Dr Pope receives research funding from the EB Research Foundation. Dr Paller serves as an investigator for Castle Creek and Lenus Pharmaceuticals and has been a consultant with honorarium for Abeona. Dr Levy serves on the advisory board for Cassiopea, Regeneron; as an

investigator for Fibrocell/Castle Creek, Galderma, Janssen, Pfizer; on the Data Safety and Monitoring Board for Novan; and as a section editor for UpToDate. Dr Lucky serves as an investigator for Lenus Pharmaceuticals and Castle Creek and on the scientific advisory board for EBRP (EB Research Partnership) and Abeona. Dr Glick serves as an investigator for Lenus Pharmaceuticals. Authors Phillips, Augsburger, and Peoples and Drs Huang, Kaplan, Khuu, Tang, Lara-Corrales, Wiss, Levin, Morel, Hook, Eichenfield, McCuaig, Powell, Castelo-Soccio, Price, Schachner, Browning, Jahnke, Shwayder, and Bayliss have no conflicts of interest to declare.

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