Cystic fibrosis year in review 2021

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Abstract

People with cystic fibrosis (CF) have an amazing outlook with the treatment availability of highly effective modulators. Unfortunately, not all PwCF are eligible for modulators leading to continued pulmonary exacerbations and advanced lung disease. Additionally, optimizing diagnosis and evaluation for CF in the newborn period continues to be an area of focus for research. This review article will work to cover articles published in 2021 with high clinical relevance related to the above topics, however due to the extensive body of research published, this review will not be comprehensive.

Introduction

Numerous publications related to cystic fibrosis (CF) were published in 2021, highlighting the amazing community around the globe helping to share research and experience. Exciting areas of research are underway; however, this review will not be comprehensive, but focus on clinically relevant highlights related to CFTR modulators, advanced lung disease, pulmonary exacerbations with clinical anti-inflammatory trials and newborn screening.

CFTR Modulators

The cystic fibrosis transmembrane regulatory conductance (CFTR) modulators have been exciting to watch over the years, with no exception in 2021, where additional advances were published, expanding eligibility to younger ages and additional mutations while also garnering a better understanding of the long-term effect on outcomes for people with CF (PwCF).

Ivacaftor

Ivacaftor (IVA) has been approved for the longest amount of time and is currently approved in the United States (US) for 4 months of age and older based on the ARRIVAL study[1]. This single arm, phase 3, multicenter study was conducted in infants between 4 and 12 months of age. Of note, based on one infant with elevated concentrations of IVA, infants under 4 months of age and 5 kilograms were not included and will be investigated in a future study. IVA was found to be safe, well tolerated, have similar pharmacokinetics to older patient populations, no adverse events, and no new safety signals. Sweat chloride (SC) decreased by a mean absolute change of -55.7 mmol/L. More exciting however, was the continued support that CFTR modulators may delay or minimize progressive exocrine pancreatic dysfunction. This was demonstrated by an elevation in fecal elastase levels (mean absolute change of 166 micrograms/g) and decreases in immunoreactive trypsinogen (IRT), amylase, and lipase levels. It is exciting to see the effects of earlier treatment with CFTR modulators as younger ages are studied.

Expanding the eligibility of IVA, a phase 3b, randomized, double blind, placebo controlled, single center, crossover study in patients over age 6 years, examined use of IVA over 8 weeks, in 39 patients with at least one copy of 3849+10kbC>T or D1152H mutation[2]. As per other studies, lung clearance index ($LCI_{2.5}$), SC, forced expiratory volume in one second percent predicted (FEV1pp) and CF quality of Life Respiratory Domain (CFQ-R) all improved; with a safety profile consistent with prior studies. Interestingly, the study

obtained patient derived rectal organoids which showed dose dependent swelling, however, individual clinical responses did not correlate with the degree of swelling. Key outcomes from this study include support for clinical benefit of IVA for these mutations and use of organoids to identify responsive mutations.

Although clinical trials provide information about the effects of IVA, an understanding of real-world, long-term use is also valuable. Four separate reports, from Canada[3], the United Kingdom (UK) [4], France[5] and the US[6] assessed IVA over time. Both the US and France conducted specific studies to observe these effects, the BRIO (cystic fi*BR* osis *I* n *O* bservation) in France (129 patients followed for 24 months post IVA initiation) and the GOAL-e2 (G511D Observational Study - Expanded to additional genotype and Extended for Long Term Follow up) (96 participants, up to 5.5 years post IVA initiation) while the UK report was an evaluation of one center (35 patients over 5 years) and the Canadian report utilized the Canadian CF Registry (144 patients over 4 years). All studies demonstrated improvements in pulmonary function, growth parameters, decreases in pulmonary exacerbations, reductions in *Pseudomonas aeruginosa*(PA), and no differences from prior reports in safety signals. In the US study, although improvements in SC were seen, there was no correlation with change in FEV1pp or body mass index (BMI) at any of the analyzed time points[6]. In the UK, an interesting analysis of IVA medication possession ratios (MPR) found an overall decline of 2.5 % per year, with those PwCF who had higher MPR demonstrating greater FEV1 change[4]. These real-world studies show continued support for the use of ivacaftor to improve a broad array of clinical outcomes with no long term safety concerns.

Lumacaftor/Ivacaftor

Lumacaftor/IVA (LUM/IVA) has also had some extended long-term data published in the past year. Two studies examined open label extension beyond the initial 24 weeks of LUM/IVA, extending to 96-120 weeks in both 2–5 [7] and 6–11 year-old participants[8]. Both studies found no changes in safety signals and sustained improvements in clinical outcomes. It is notable that only 9% of the 2–5-year-old participants had respiratory adverse event, especially in light of 2-5 year olds being a common age for early transient wheeze and older participants ([?] 12 years) in the LUM/IVA studies having respiratory adverse events as a common cause of discontinuation in prior studies[7]. Especially in the 2–5-year-old age group, where no other CFTR modulator is available for PwCF homozygous for F508del, it is reassuring to see that extend use continued to be safe and have improved clinical outcomes.

Two countries, the US[9] and France[10], examined effects in a more real world setting for 12 and 24 months respectively. The US study, PROSPECT, evaluated 196 participants, over age 6 years[9], while the French looked at 40 participants between ages 12 and 18 years[10]. As with other real-world studies, no changes in safety signals, SC decreased, and BMI increased. Of note, however the French participants had an increase in FEV1pp[10], while the US study did not, mentioning that US participants had a higher starting FEV1 compared to the phase 3 clinical trial[9]. Exacerbations were not improved in either study, while the US found no differences in hospitalizations, or PA infection[9] and the French analysis found no changes in oral or IV antibiotic usage or CT scan evaluations[10]. Improvement in FEV1pp was noted to have an inverse correlation with age at LUM/IVA initiation[10]. Neither study found a correlation between changes in SC and FEV1pp, BMI percentile or BMI[9,10]. This lack of correlation with SC was also seen in a single center study of 160 participants over six months. Of interest, there was a larger decrease in SC in females (-28.6 mmol/L) compared to males (-18 mmol/L)[11]. A sub study of PROSPECT examined mucociliary clearance and found no significant change, except in cough assisted clearance[12]. Both evaluations support LUM/IVA as a beneficial medication, thus, in countries where LUM/IVA is the only available CFTR modulator, clinical use is valuable, however, LUM/IVA is less effective and thus if other highly effective modulators are available, their use may be of more benefit to PwCF.

LUM/IVA has also seen expanded eligibility. In vitro, the mutation A455E has shown to be responsive to LUM/IVA, thus clinical outcomes were evaluated in a randomized, double blind, placebo controlled, multicenter, phase 2 study, in an 8-week crossover design in 20 participants[13]. The medication was safe and well tolerated, however, the primary endpoint was not met, with only a mean absolute change in FEV1pp of 0.1%, decrease in SC of -7.8 8mol/L and a mean absolute change from baseline change in CFQ-R of 3.5

points. Participant derived organoids showed concentration dependent swelling to LUM/IVA, however, in this small group of participants, there was no correlation with clinical response[13]. Similar to the study of organoids in IVA alone, this study adds to the use of organoids as a marker of modulator responsiveness, although in this instance, not correlated with clinical outcomes.

Continuing to work to understand the effect of LUM/IVA in populations that were not included in the original clinical trials were reported this year. One study evaluated adults over one year who had advanced lung disease ((ALD) defined as FEV1pp <40%)[14]. Of the 24 who reached the one-year time frame, a decrease in pulmonary exacerbations requiring hospitalization, days of hospitalization, IV antibiotic days, improvements in FEV1pp, and BMI were seen. No apparent clustering of improvement was seen, such that those with the greatest reduction in health care utilization were not the ones with the greatest stabilization of FEV1pp or greatest improvement in weight. Again, the importance of this study extends to PwCF and ALD in countries where no other CFTR modulator is available allowing them to benefit.

Another population not studied in the clinical trial, PwCF and CF related liver disease (CFLD), LUM/IVA was examined in 28 PwCF over age 12 (4 with multinodular liver disease and portal hypertension, 19 with CFLD and 5 without CFLD)[15]. Biomarkers of liver disease were all decreased after 12 months of treatment, while ultrasound findings varied (19 unchanged, 3 with resolution, and one with increased echogenicity). No correlation was seen between biomarkers and ultrasound findings.

Beyond new populations, additional effects on clinical outcomes have come to light. For example, changes in fat soluble vitamins. In 45 patients, over age 2 years who had treatment with LUM/IVA for one year, the following was seen, no changes in Vitamin D levels or Retinol, an increase in international normalized ratio (INR), and a decrease in Vitamin E[16]. By two years of treatment, there were no additional changes in Vitamin D or INR, yet retinol increased, and Vitamin E decreased[16].

The clinical outcome of pancreatitis has also been associated with modulator use. A case series of 5 PwCF with pancreatic insufficiency, demonstrated increased risk of acute pancreatitis after use of modulator (IVA or LUM/IVA)[17]. In contrast, using MarketScan, a database of inpatient and outpatient health care covering over 200 million individuals, an overall reduction in acute pancreatitis in both pancreatic sufficient and insufficient patients was seen after introduction of CFTR modulators[18]. Although these reports conflict, clinicians should be aware of possible pancreatitis in PwCF.

Tezacaftor/Ivacaftor

As with the other modulators, tezacaftor/IVA (TEZ/IVA) has also had explored in additional populations, which have published this past year. In ages 6 to 11 years (homozygous or heterozygous for F508del mutations), 66 participants were studied in a phase 3, randomized, double blind, placebo-controlled trial over 8 weeks[19]. No new safety concerns were seen. Statistically significant changes were seen in SC, $LCI_{2.5,}$ and FEV1pp, although no changes were seen in CFQ-R, growth parameters, or fecal elastase. Evaluating PwCF with one copy of F508del, and a gating mutation known to be responsive to IVA who were studied in a phase 3, randomized, double blind, placebo control, parallel group study of 153 participants, over age 12 years[20]. Participants received IVA for 4 weeks followed by either continued IVA or change to TEZ/IVA over 8 weeks. No clinically meaningful benefit was seen for FEV1pp, CFQ-R, or SC. Extension of TEX/IVA usage was examined in participants from prior TEZ/IVA trials, over age 12 years (EXTEND study). Although, many participants withdrew due to non-efficacy (especially in those heterozygous for F508del), 682 participants completed 96 weeks. No new safety signals were elucidated, however, a relative reduction in rate of decline of FEV1pp of 61.5% was seen for participants homozygous for F508del when compared to historical controls[21]. Those PwCF who had discontinued LUM/IVA due to respiratory related signs and symptoms, were evaluated using TEZ/IVA. In the 94 participants who completed the study over 56 days, the respiratory adverse event rate was 14% compared to placebo rate of 21.3%, with no discontinuations of TEZ/IVA[22]. Finally, an alternate analysis, using the CF Foundation Patient Registry(US CFFPR), statistical modeling explored the difference in FEV1pp between those PwCF exposed to smoke (self-reported) compared to those with no exposure[23]. It is noteworthy that at baseline, PwCF who were exposed had an almost 8% lower FEV1pp.

After initiation of TEZ/IVA those exposed had a greater decline in FEV1pp compared to those not exposed to smoke, essentially nullifying the effects of TEZ/IVA. Thus, the published reports for TEZ/IVA this year show benefit for younger ages (6-11 years), TEZ/IVA longer term (mainly homozygous F508del) use and reduced respiratory adverse events, however, for those with only one copy F508del or those exposed to smoke, minimal benefit was seen.

Elexacaftor/Tezacaftor/Ivacaftor

Elexacaftor/TEZ/IVA (ETI) is the most exciting highly effective modulator available in the US since late 2019. In the past year, there were two phase 3 trials reported: one in 258 participants over age 12 with one F508del mutation and a gating or residual function mutation already on IVA or TEZ/IVA and transitioned to ETI over 8 weeks[24] and a second trial over 24 weeks in 6-11 year old participants with two copies F508del or a F508del and a minimal function mutation[25]. Neither study had changes in safety signal, and both demonstrated improvements in FEV1pp, SC, CFQ-R. Additionally, growth parameters were improved in the 6–11-year-old evaluation. It is important to note those PwCF who were already on IVA or TEZ/IVA, demonstrated a between group difference of 3.5% in FEV1pp, thus showing additional improvement with ETI above IVA or TEZ/IVA. Moreover, indication for ETI was shown in a patient with N1303K/E193X mutations who had improvement in the lab, followed by improvements in FEV1pp, BMI, CT scan of the chest, and sinus imaging[26]. Thus, ETI has been shown to be effective in those transitioning from other modulators, the 6–11-year-old age group and an individual with the N1303K/E193X mutation.

The long term effects of ETI in PwCF over 12 years of age, was reported in three studies, a post approval multicenter study at four German centers[27], the PROMISE study, the US's post approval, real -world observational study[28], and an interim analysis of the open label extension study for participants from the clinical trials[29]. Timing differed between the studies, from 8-16 weeks post ETI initiation[27], through 6 months of ETI use[28], or once all participants completed 24 weeks in the clinical trials[29]. None of the studies showed additional safety concerns. Participants had improvements in FEV1pp, BMI, and SC (if not previously on ETI as part of clinical trial). It is also important to note that patients already on a modulator who transitioned to ETI had additional improvements in clinical outcomes (FEV1pp increased 6.1-9.2%, SC decreased -23.9 to 43.4 mmol/L)[28]. Both the PROMISE and the German evaluations demonstrated a correlation between SC changes and FEV1pp[27,28]; whereby the PROMISE reported for every 10-point decrease in SC there was a 0.89% increase in FEV1pp[28]. As with prior findings with LUM/IVA, the PROMISE study found a larger decline in SC in females[28]. Improvements in nasal potential difference (NPD) and intestinal current measurement (ICM), with improvements up to 40-50% of normal CFTR levels, in the German studies[27]. Changes in NPD did not correlate with FEV1 or BMI; however, ICM was noted to have a weak correlation. From PROMISE, self-reported use of pulmonary maintenance medications usage decreased by 6% for dornase alpha, 9.8 % for hypertonic saline, 9.1% for azithromycin, and 34% for inhaled antibiotics.

Expanding usage of ETI to population not studied in the original clinical trials, use for PwCF and ADL, based primarily on a FEV1pp <40% or referral/evaluation for lung transplantation demonstrated improvements in FEV1pp[30-34], BMI[31-34], CFQ-R[32,34], and decreases in antibiotic use/exacerbations[32,34]. Where reported, oxygen use was decreased/eliminated[32,34], with one study demonstrating a decrease in noninvasive ventilation use[31]. Most importantly, transplantation status primarily changed in a positive direction, with decreases in transplant related discussions, referrals, and wait list numbers[30-33]. Overall, lung transplants decreased by 56.4% in France, attributed to ETI, as the COVID related decrease in non -CF indications only dropped 26.4%[31]. When data from prior to 2002 was comparted to late 2020/early 2021, France saw a tenfold reduction in transplantation rate[35]. Similarly, according to an analysis of the International Society of Health Lung Transplantation, between 1990-2017, there was a decrease in CF as an indication for lung transplantation[36]. Therefore, the use of highly effective modulators have changed the landscape of lung transplantation.

Effect on Women's Health

Evidence of the increase in fertility in women on modulators is in abundance. Using the United Kingdom CF

Registry through 2017 (prior to ETI availability), the overall pregnancy rate in PwCF was 3.3 times lower than the general population; however, after introduction of CFTR modulators, the pregnancy rate increased 1.5 times in those with G551D mutations[37]. A retrospective review at two centers also demonstrated an increase in pregnancies after initiation of ETI, noting that many of those females previously had infertility or subfertility issues[38]. Based on this increase in fertility, an increased understanding of the needs of PwCF in regard to reproductive goals and family planning was explored[39], including in the adolescents population[40]. The pregnancy outcomes were reported in case series for 52 pregnancies in women with CF on ETI[41,42]. The clinical course varied, some opting to stop ETI, while others continued, but complications were minimal with none felt to be related to ETI. Assessing 3 mother infant pairs, ETI was found to be measurable in the cord blood and breast milk[43]. Separately, infants evaluated during lactation had no complications, however only two were formally assessed for cataracts. One of the most exciting reports in the past year, was the case report of an infant with CF born to a mother with CF who was on ETI during pregnancy[44]. The infant had a false negative NBS, however genetic testing revealed two copies of F508del. The infant was breast fed while mom continued ETI. Additional testing revealed a SC of 60-67 mmol/L, normal fecal elastase as assessed monthly, normal ophthalmologic exam for cataracts, and no respiratory concerns. Overall, the improvements in women's health are remarkable and it is truly exhilarating to hear about the outcome for the infant with CF who received ETI while in utero/through breast milk.

Effect in Transplant

PwCF who have had a transplant, were not included in the original trials for modulators, however many have started to explore the outcomes. A case series of 9 PwCF who had a lung transplant and started on ETI demonstrated stability or improvement in their graft function, improvements in BMI, and no one required changes in immunosuppression dosing[45].

PwCF who had liver transplants, the use of ETI has been shared in multiple case reports, showing increased/stable FEV1pp and BMI[46-48]. Based on the known side effects to the liver with CFTR modulators, the starting dose of ETI ranged from full dose, AM dose only, and half of the AM dose[46-48]. The ultimate dose remained at full dosing regimen, only the AM dose, and some discontinued ETI[46-48]. For those with a reduced final dose, it was for several reasons, including clinicians never increasing, elevation in liver function tests (LFTs), and one patient with tacrolimus toxicity and acute kidney disease. Laboratory monitoring varied with each patient but included LFTs and immunosuppressive medication levels. Changes in dosing based on the interaction between immunosuppressive medications and the CFTR modulator also varied, no change in dose was seen in one PwCF on mycophenolate, while those on tacrolimus/sirolimus required no changes, reductions and in only one case an increase in dosing. Overall, the patients showed improvements while being monitored closely for changes in immunosuppressive dosing and LFTs. Therefore, these cases highlight additional benefits in patients who have had transplants, with the importance of careful monitoring.

Multisystem Effects

As is well known, CF effect more than the lungs, thus there were several notable reports related to ETI use and multisystem alterations. Presence of *Mycobacterium abscessus* infection for over 12 years, was no longer seen in culture in one PwCF after starting ETI[49]. Vitamin levels were altered, with vitamin E levels increasing 12 months after ETI in a review of 76 patients[50] and 2 reported cases of papilledema with elevated Vitamin A levels[51]. Mental status changes (mainly mental fogginess) were seen in a case series of 6 patients, all stopping the medication withing 3 months of initiation, despite attempts at altered dosing regimens[52]. Glycemic effects were examined in 24 patients with and without CF related diabetes demonstrating a reduction in glucose parameters from continuous glucose monitoring and no one showed evidence of hypoglycemia[53]. Biliary disease was seen in 7 patients at two adults centers, with 6 patients requiring cholecystectomy[54]. Although the additional effects of ETI are still being determined, some of benefit, but some of concern, thus additional care/concern should be undertaken when monitoring our patients on modulators.

Advanced Lung Disease

The CF community is incredibly fortunate to have highly effective modulator therapy, however, there con-

tinues to be a role for care of PwCF and ALD. The French 3-year prognostic score for death or lung transplantation, previously verified by Canada, has also been verified using the US CFFPR and United Network for Organ Sharing (UNOS) data, thereby providing useful information to inform discussions with PwCF and ALD[55].

Once PwCF have ALD, many varied treatment modalities are utilized, such as noninvasive ventilation (NIV). A review of NIV over 9 years (2011-2019) at one center, demonstrated increases in lung function, BMI, and 47% of the patients survived/made it to transplant during the 9 years studied[56]. Pre transplant characteristics of PwCF with ALD can affect post-transplant outcomes, therefore understanding these factors is important to allocate the rare resources. Data from UNOS on 3881 patients, over age 18, who were transplanted between 1992-2016 was assessed to evaluate pre transplant factors[57]. Post-transplant survival was higher for those [?]30 years old (9.47 years vs. 5.21 years in 18-29 years of age). Private insurance was also associated with increased survival. Using the Scientific Registry of Transplant Recipients, in 2573 patients (>18 years, 2005-2018), transplant centers with accreditation by the CF Foundation had a greater survival (7.8 vs 4.4 years) and a 33% reduction in graft failure despite no difference in Lung Allocation Scores or volume of transplantations[58]. Using data from a combination of UNOS and the CFF PR, adults with ALD who had a BMI [?]17 kg/m2 had lower likelihood of referral, listing for transplantation, and a higher risk of death without transplantation, although regional variation did exist[59]. These epidemiologic evaluations provide clinicians with strong evidence to continue to work to optimize health while referring for transplantation to achieve the most optimal long-term outcome.

Once patients are critically ill, many centers use mechanical ventilation and/or ECMO as a bridge to transplantation. UNOS data from 2015 – 2020, in patients 12 years of age and older, showed that of the 1064 patients requiring ECMO, 13% had CF[60]. There was no difference in likelihood to receive lung transplantation while on ECMO for PwCF compared to those with obstructive lung disease, or interstitial lung disease. A separate analysis of UNOS, evaluated 68 patients in a younger cohort (under 20 years of age), over 2004 – 2019, and found 42.7% of those requiring ECMO as a bridge to transplantation, had CF[61]. In this younger cohort, use of ECMO as a bridge had 2-3 times odds of mortality prior to discharge compared to those on a ventilator. Furthermore, pulmonary hypertension and black race were independent predictors of mortality. Once discharged, however, no difference was seen in 1- or 5-year mortality or re-transplantation rates. Overall, the mortality rate with ECMO has decreased due to improvements in ECMO care, from 75% in 2010, to 33% in 2018. In one center's comparison of invasive ventilation versus ventilation plus ECMO as bridges to transplantation, no differences were seen for mortality prior to discharge, or at 1, or 5 years, however, those on ECMO had longer length of stay[62]. The information related to ECMO is encouraging as there have been improvements in care leading to decreases in mortality and therefore increasing the potential benefit of using ECMO as a bridge to transplantation.

Outcomes for transplantation vary, with some requiring re-transplantation. Using UNOS data from 2005 – 2020, 52% of 534 adults who underwent a repeat double lung transplant had CF[63]. Re- transplantation was associated with more renal and cardiac disease, increased rates of reintubation, longer times on ventilator post-transplant, longer length of stay, and decreased 5-year survival compared to initial double lung transplantation.

Differences in outcomes have also been seen between the US and Canada, such that between 2005 and 2016, PwCF and ALD in the US had an equal chance of death or transplantation, while those in Canada were two times more likely to receive a transplant than die[64]. Lung transplantation rate was lower in the US. A separate analysis over the same time found that differences in death without transplantation and post-transplant survival explained about 30% of the survival gap between the two countries[65]. Overall, lung transplantation has come a long way in care and outcomes, and through continued understanding of variations, care teams can work to provide optimal systems to ensure best possible outcomes.

Pulmonary Exacerbations (PEx)

Despite the use of CFTR modulators, PEx still occur, and research related to optimizing outcomes has been

published this year. Standard of care for PEx is to treat with two anti- PA antibiotics, however a recent analysis using used both the CFF PR and the Pediatric Health Information System (PHIS), one versus two anti-PA antibiotics, was analyzed in over 2500 exacerbations[66]. Investigating the year 2007-2018, in PwCF 6-17 years of age, no differences were seen between one or two anti-PA antibiotics in pre or post exacerbation FEV1pp, odds of returning to [?]90% of baseline FEV1pp within three months, or time to next PEx requiring IV antibiotics. Therefore, additional prospective studies are indicated to determine use of only one anti – PA antibiotic would be beneficial by limiting antibiotic exposure while remaining clinically effective.

In addition to using FEV1pp as a marker of PEx improvement, the Chronic Respiratory Infection Symptom Score (CRISS) was evaluated in patients as part of the Standardized Treatment of Pulmonary Exacerbations (STOP)- Observational trial. The median baseline CRISS score was 49, and 93% of patients with PEx had a decline of [?]11 points (the minimal clinical important difference), thus CRISS was felt to be a useful efficacy endpoint[67]. Further analysis of the STOP cohort found the only difference between males and females was 13% more IV antibiotics treatment days for females[68].

Optimal length for PEx was the subject of the STOP-2 trial[69]. If a participant had at least 8% improvement in FEV1pp and 11-point decrease in CRISS by day 7-10 of exacerbation, then 10 days of IV antibiotics was not inferior to 14 days. For those participants without an improvement at 7-10 days, 21 days of IV antibiotics was not superior to 14 days. As a sub study, the participants in STOP-2 study, C-reactive protein (CR) was assessed at start of antibiotics, 7-10 days into the PEx and two weeks after end of treatment, however levels were found to be variable and thus the authors concluded that the utility of CRP as a biomarker for PEx treatment response is limited[70]. Continued understanding of ideal methods for PEx treatment will remain of use since modulators have not eliminated PExs for PwCF.

Newborn Screening

CF newborn screening (NBS) is a pertinent area of research interest, with work in the past year published related to optimization of both the diagnostic pathway as well as clinical care delivery for both CF and CF related metabolic syndrome/CF screen positive inconclusive diagnosis (CRMS/CF-SPID) patients.

NBS has been available in every state in the US since 2010 and other countries with variable initiation dates. NBS varies by location, but an ongoing awareness of each state/country's outcomes are critical. The European CF Society Neonatal Screening Workgroup published key performance outcome parameters with clear definitions to help assess and monitor NBS programs[76].

Several locations have shared their experiences with valuable lessons shared. In a report from Colorado, 8 cases were missed over 5 years[71]. Upon data review, use of a fixed cut off for IRT (60 ng/ml) explained the missed cases, as over time, IRT values gradually decreased. To account for known variations seen with IRT levels, Colorado changed to a 96%ile floating cutoff and 50ng/ml fixed cutoff, which would have prevented these missed cases. New York state changed to an IRT/DNA sequencing algorithm in 2017 and demonstrated the ability to use customizable targeted sequencing of all clinically relevant CFTR variants in an effort to decrease false positive testing[77]. In Germany, to reduce identification of carriers, the use of IRT, pancreatitis associated protein (PAP) with an IRT dependent safety net was compared to IRT-DNA method over the years 2008-2016. The PAP method had good sensitivity and the advantage of detecting no carriers, however had a low positive predictive value (PPV), leading authors to conclude that DNA may be necessary as a third tier to improve the PPV[78]. Poland identified 11 patients with a false negative NBS, with many due to lack of SC testing in subsequent siblings, infants with meconium ileus, and patients with symptoms consistent with CF despite normal NBS results[79]. These reports highlight the role of ongoing data monitoring, false negatives in NBS including from known variation in IRT levels, advancing to CFTR sequencing and use of PAP in NBS for CF.

The use of palmar aquagenic wrinkling is a possible screening method for CF[80]. To test this hypothesis, children less than 2 years of age were examined. Time to wrinkle was much shorter in PwCF compared to carriers/controls and correlated with IRT, SC levels, and CFTR mutations. These results may be of benefit in countries with financial and geographic limitations to NBS implementation, whereby use of time

to wrinkle can serve as a screening tool to determine those patients who need earlier referral for diagnostic testing.

Once a NBS is positive, patient care pathways are important to optimize outcomes. The CF Newborn Screening Genetic Counselling Workgroup, a cross disciplinary expert group, recommends that centers ensure access to a trained genetic specialist for parents of infants with a positive NBS[74].

The first 9 years of NBS in the US were reviewed in 6,354 infants from the CFF PR[73]. The first event (defined as earliest SC test, clinic visit or hospitalization) for NBS positive infants was withing the first 30 days for 77% yet 10% still had their age at first event at >60 days. The percent with age at first event beyond 60 days has decreased over the 9 years, from 11% in 2010-2012 to 7.8% in 2016-2018. It is critically important for long term clinical outcomes to have an earlier age at first event, especially with the importance of early nutrition. It was notable that 40% of these infants had a weight for age of <10% at their first visit, demonstrating early evidence of nutritional concern. This evaluation of NBS over time in the US serves as a reminder to clinicians of the importance of early diagnosis/evaluation (prior to 30 days of age) and the critical importance of nutritional monitoring

Long term follow-up regarding the benefits of NBS were explored in PwCF diagnosed as part of the Wisconsin CF Neonatal Screening Project who have been followed up to 26 years[75]. No difference in mortality was seen at age 25 (89% of screened vs. 85% diagnosed symptomatically). In those diagnosed by NBS, the FEV1pp rate of decline was greater between ages 7-26 years (-1.76 vs. 1.43% per year), however, was concluded to be partially due to earlier PA acquisition. The authors concluded that CF NBS was not significant enough to improve longitudinal lung function decline.

CRMS/CF-SPID

Those infants with a diagnosis of CF related metabolic syndrome (CRMS) or CF screen positive inconclusive diagnosis (CFSPID) were included in an updated guidance document from the European CF Society Neonatal Screening Working Group[81]. Global harmonization of the definition now has these infants referred to as CRMS/CFSPID. Some pertinent guidelines worth highlighting include, checking the CFTR2.org or CFTR-France database regarding classification and characterization of the mutation to assess for updates in classification. SC tests should be completed at initial assessment and repeated at 6 months, 2 years, and 6 years of age. At six years of age, a more extensive evaluation is recommended with lung clearance index or spirometry and chest imaging. If the results of these assessments demonstrate normal growth, lung function, imaging, and SC test <30, then it is unlikely they will convert to CF and the patient can be either a) discharged from CF care b) re-assessed again at age 14-16 years of age, or c) continue care with specialist. Further details regarding care recommendations are outlined in the publication.

Some of the most recent guidelines were informed by various research to better understand the outcomes for patients with CRMS/CFSPID. In Indiana, patients with one mutation on NBS and a SC in the indeterminate range were followed to assess overall potential for fulfilling full diagnostic criteria for CF[82]. Subsequent diagnosis with CF, occurred in none of the infants with a SC from 30-39 mmol/L, one out of 31 infants with SC 40-49 mmol/L, and 61% of those with SC from 50-59%. An Italian survey of centers revealed that 5.3% of the CRMS/CFSPID were re-classified over time as CF[83]. Specifically, in 50 infants with CRMS/CFSPID, who had early and frequent SC testing, a definitive diagnosis (CF, CF carrier, healthy infant) was found in 22% by 8.5 months, however CF was only seen in 1%[83]. In contrast to the Indiana data, the Italian analysis had 8 patients with a SC value between 50-59 mmol/L and only one progressed to a diagnosis of CF. A separate single center study found 6% of CRMS/CFSPID patients were reclassified as CF over the course of 12 years[84]. In Canada, 115 children with CRMS/CFSPID over ˜7 years were assessed, and CF was diagnosed in 21%[85]. Those infants with a SC that was [?]40mmol/l were found to have a ten times higher hazard of having a CF converting SC level. The major message in common for these reports is the importance of clinical vigilance, repeated SC testing, and the fact that most infants will remain with a diagnosis of CRMS/CFSPID.

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