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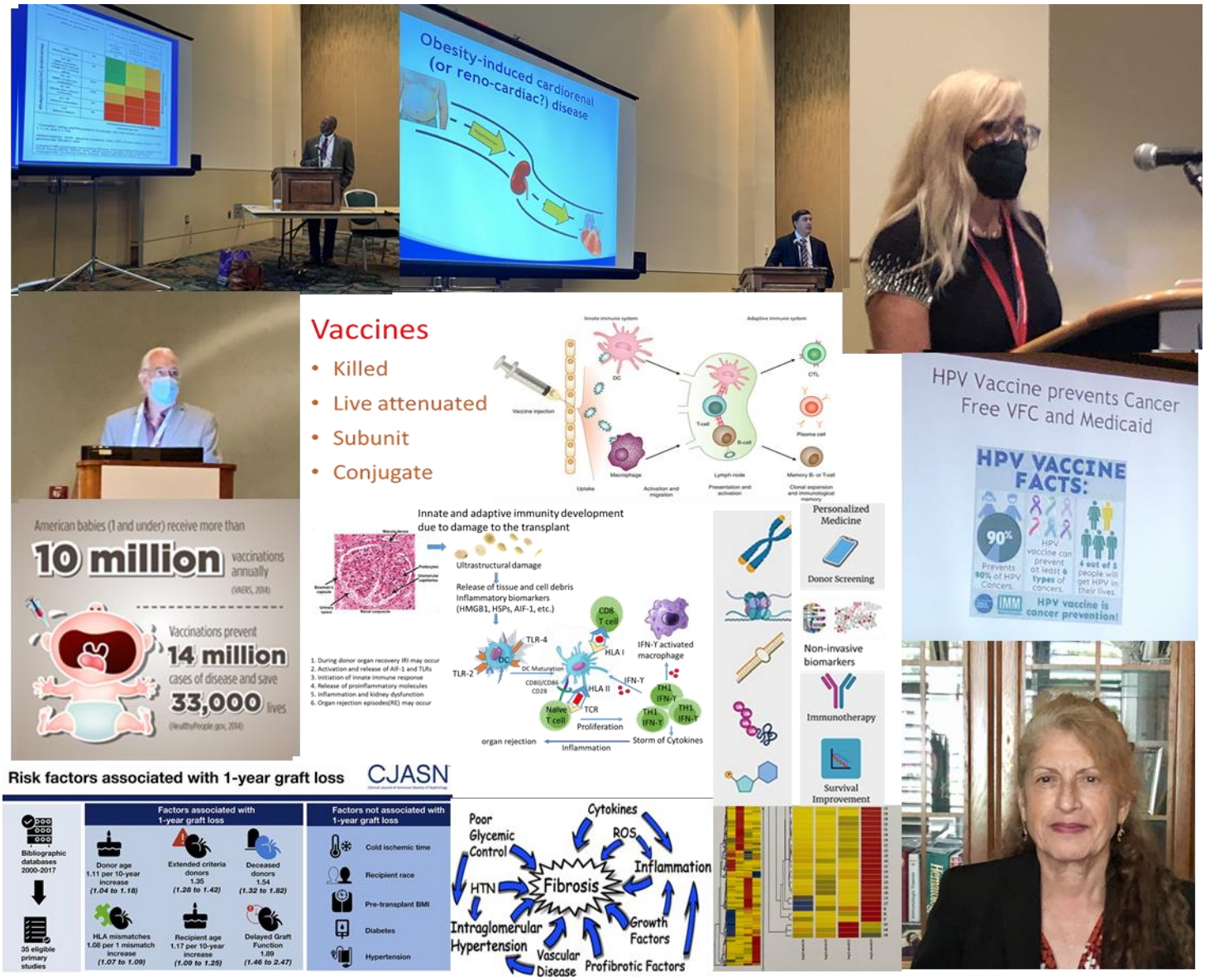
Special Edition

Population Health Disparities and Disease:

In the era of precision medicine



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MAS Special Chapter Prelude

This special issue of the Journal of Mississippi Academy of Sciences (JMAS) is a collection of topics relevant to population health and diseases. The characteristics of this collection are multitudinous, explicitly has been described with the goal to provide the state of art scientific and disease associated materials to our readers. In this scenario:

1. Dr. Errol Crook, nephrologist and his colleague, currently at University of South Alabama, have made a great contribution describing progress in diagnosing and treating chronic kidney disease.
2. Dr. Michael Hall, cardiologist and his associates, at University of Mississippi Medical Center (UMMC), discussed reasons that an individual with hypertension may develop heart failure and the impact of racial disparities on hypertension and heart failure. Prevention and treatment strategies are discussed.
3. Dr. Larry McDaniel, microbiologist at UMMC, highlighted the importance of vaccine and vaccination throughout the last century and have emphasized on protective immune responses after vaccination.
4. Carolann Risley, Associate Professor at UMMC, School of Nursing, and her collogues, discussed prevalence of Human Papillomavirus (HPV)-associated cancers in population and reviewed the engagement of Mississippi state department of health (MSDH), National Institute of Health (NIH) and UMMC towards increase and improve community awareness in the state.
5. Dr. Olga McDaniel, Scientist and Emeritus Professor of Surgery, described current understanding of innate immune response in organ transplantation and the application of molecular biomarkers in prediction and diagnosis of early clinical outcomes.

The contents of this special issue are meant to engage the reader and provide insight into areas that affect population health in the era of precision medicine. Finally, it is important to recognize the hard work of the contributors in this issue.

Progress in Diagnosing and Treating Chronic Kidney Disease

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ABSTRACT

Chronic kidney disease (CKD) is a leading cause of death in the United States and has a high incidence and prevalence in Mississippi and other Southeastern US states. While 15% of US adults are estimated to have CKD, unfortunately, the majority of people with CKD are unaware of their diagnosis. CKD accounts for a disproportionate share of Medicare spending with much of this spending due to the cardiovascular complications associated with CKD. Health disparities are significant in CKD with ethnic minority populations, particularly African American and Hispanic, and those in poverty having significantly higher rates of CKD. Diabetes Mellitus (DM) is the most common cause of CKD, and it is known that blood pressure control, use of renin-angiotensin inhibitors, and blood sugar control slows the progression of CKD in patients with DM. Despite this knowledge the incidence and prevalence of CKD continues to increase and many of those who achieve blood pressure and blood sugar control will still have progression of CKD. We review progress in earlier detection of those at risk for CKD, particularly due to DM, and progress in determining who may be at higher risk of progression. In addition, we outline newer pharmacologic therapies that slow the progression of CKD and have additional benefits of preventing the cardio vascular complications of CKD

KEY WORDS: African American (AA), Chronic Kidney Disease (CKD), Diabetes Kidney Disease (DKD), End Stage Kidney Disease (ESKD), Health Disparities (HD), APOL-1

INTRODUCTION

Chronic Kidney Disease (CKD) (see Table 1 for definitions) is a common cause of death and disability in the United States. Approximately 1 in 7 adults has chronic kidney disease (CKD) (CDC 2021a), however most of these 37 million Americans are unaware of their diagnosis. Nine out of 10 adults with CKD, and even 2 out of 5 with severe CKD do not know they have it (Bowe et al., 2018, USRDS 2021, CDC 2021a). The financial costs of CKD are disproportionate to its prevalence. For example, in 2019, total Medicare fee-for-service costs for CKD beneficiaries without End Stage Kidney Disease (ESKD) accounted for almost a quarter of the Medicare fee-for-service expenditures ($87 billion), but only 13.6% of Medicare fee-for-service clients of age >66 years had CKD (USRDS 2021). The impact is much greater when we consider that ESKD, a consequence of the progression of CKD, added an additional $37.3 billion in Medicare cost that same year (USRDS 2021). Unfortunately, the prevalence of ESKD has doubled over the last 20 years (Burrows et al., 2022).

There are important disparities in how CKD/ESKD impacts various populations across the US (Table 2). Southeastern US States, Alabama (AL) and Mississippi (MS) in particular, are consistently among the states with the highest rates of CKD. As a group, Alabama, Mississippi and Tennessee, have the highest incidence rate and third highest prevalence rate of ESKD nationwide (USRDS 2021). In addition, there are considerable inequities in CKD/ESKD across ethnic groups. African Americans (AA) in particular suffer disproportionately with CKD/ESKD. AA population living in MS and AL are 4 times more likely to develop kidney disease than non-Hispanic whites living in those states (Benjamins et al., 2022, USRDS 2021). The high rates of Diabetes

Mellitus (DM), hypertension and obesity among AAs contribute, in part, to the high rates of CKD/ESKD seen in AAs in the Southeastern US and the nation at large. However, the higher prevalence rates of those diseases alone do not account for the much greater rates on CKD / ESKD experienced by AAs (CDC, 2021b, Crook, 2002). In fact, the Southeastern US can be referred to as the kidney disease belt as much as it is referred to as the stroke belt.

Table 1. Clinical Characteristics and Definition of CKD, DKD, and ESKD

Chronic Kidney Disease (CKD)	● CKD is defined as abnormalities in structure and/or function of the kidney for at least 3 months. ● Chronic kidney disease is categorized by stages (Stages 1-5). ● Higher stages indicate worse kidney function. (Stage 2- Mild, Stage 3 – Moderate, Stage 4- Severe, Stage 5-ESKD (see below)). ● Evaluation of function is based primarily on the glomerular filtration rate (GFR) and level of protein in the urine. ● In many studies GFR<60ml/min/1.75m^2 is considered chronic kidney disease.
Diabetic Kidney Disease (DKD)	● Kidney disease caused primarily by diabetes, also referred to as diabetic nephropathy. ● It is typically characterized by the presence of albumin in the urine in a patient with DM. ● DKD is the number one cause of CKD and ESKD in the U.S. ● At times DKD is used when referring to any patient with DM who has kidney disease, regardless of whether DM is the primary cause of the kidney disease.
End Stage Kidney Disease (ESKD)	● The final stage of CKD at which the kidneys no longer retain meaningful function. ● At this stage patients will eventually require lifelong dialysis or kidney transplant to sustain life.

References: KDIGO 2012

Table 2: Factors Associated with Health Disparities in CKD / ESKD

Factors	Characteristics
Geography	Increased incidence, prevalence and mortality rates in Southeast US and in urban areas with high density of African Americans and Hispanics.
Ethnicity	Compared to non-Hispanic Whites all ethnic groups have higher prevalence rates of ESKD. African Americans have CKD/ESKD rates that are up to 4 times higher than non-Hispanic Whites in the US. American Indian / Alaska Natives have had decreased in their incidence rates.
Educational Attainment	Individuals with lower education attainment are at higher risk for CKD and ESKD. Almost 20% of individuals with ESKD are without a high school diploma.
Poverty	Poverty rates are higher among patients with CKD. Over 27% are underinsured or uninsured. Uninsured -11.7% Medicaid - 16.1%
Low Health Literacy	Patients with severe CKD / ESKD are more likely to have low health literacy compared to the general population.

References: Benjamin et al., 2022, Burrows et al 2022, CDC 2021a, USRDS 2021

Challenges in Reducing the Impact of CKD

Despite advances made over the last several decades in understanding the pathophysiology of several kidney diseases, there continues to be an increase in rates of CKD and ESKD in the US. Diabetic kidney disease (DKD) and hypertension are the first and second most common causes of CKD respectively (USRDS 2021). Control of blood pressure is the most important intervention to decrease the progression of CKD to ESKD, and it is recognized that inhibition of the renin angiotensin aldosterone system (RAAS) is an important component of the blood pressure lowering regimen (Crook, 2002). However, despite adequate blood pressure reduction with inclusion of RAAS inhibition, there are many patients with CKD, particularly those with DKD, that have progression of their disease.

Perhaps the greatest challenge in CKD is understanding and disrupting the link between CKD and cardiovascular disease (CVD) (Crook and Washington 2004, Afkarian et al., 2013). Higher CVD rates and worse CVD outcomes have long been observed in patients with CKD. In a cross-sectional study, Go et al., 2004, demonstrated that CVD risk increased as glomerular filtration rate (GFR), a measure of kidney function) worsened (Go et al., 2004). In addition, our group demonstrated that incident CVD events increased as persons with established CKD had progression of their kidney disease to higher CKD stages. Among predominantly AA patients with DM and CKD, worsening from CKD stage 2 to CKD stage 3 or from CKD stage 3 to CKD stage 4 was associated with increased risk of CVD events, particularly heart failure and stroke (White and Crook 2004).

To reverse the increase in incident ESKD observed over the last 20 years, it is critical that healthcare providers be better able to predict which patients will have progression of their CKD and, who among patients with CKD, is at higher risk for CVD events and death. We review the development of some novel tools that enhance the ability to predict who is at greater risk for CKD, particularly among high-risk groups like patients who are AA and those with DM and hypertension. In addition, we review some advances in therapeutics that have reduced the likelihood for progression of DKD and have lowered the risk for CVD among patients with CKD.

Advances in Reducing the Progression of CKD

The pathophysiology underlying the development and progression of CKD is a complex interplay between several factors promoting maladaptive changes, tissue and architectural remodeling, and cellular injury, aspects of which are yet to be fully understood (See Figure 1) (Matovinović 2009). The pathophysiologic pathways that contribute to the development of DKD are perhaps among the most studied. The initial event is felt to be metabolic derangements associated with elevated blood glucose and/or hemodynamic aberrations, perhaps due to elevated blood pressure, resulting in vascular dysfunction that is not limited to the kidney. These initial events are followed closely by an increase in inflammation and injury to important structures of the renal glomerulus: mesangial expansion due to fibrosis, endothelial injury, and podocyte injury (see Table 3) (Barrera-Chimal and Jaisser 2020).

Figure 1. A summary of immunological and clinical outcomes contributing to the development of kidney disease.

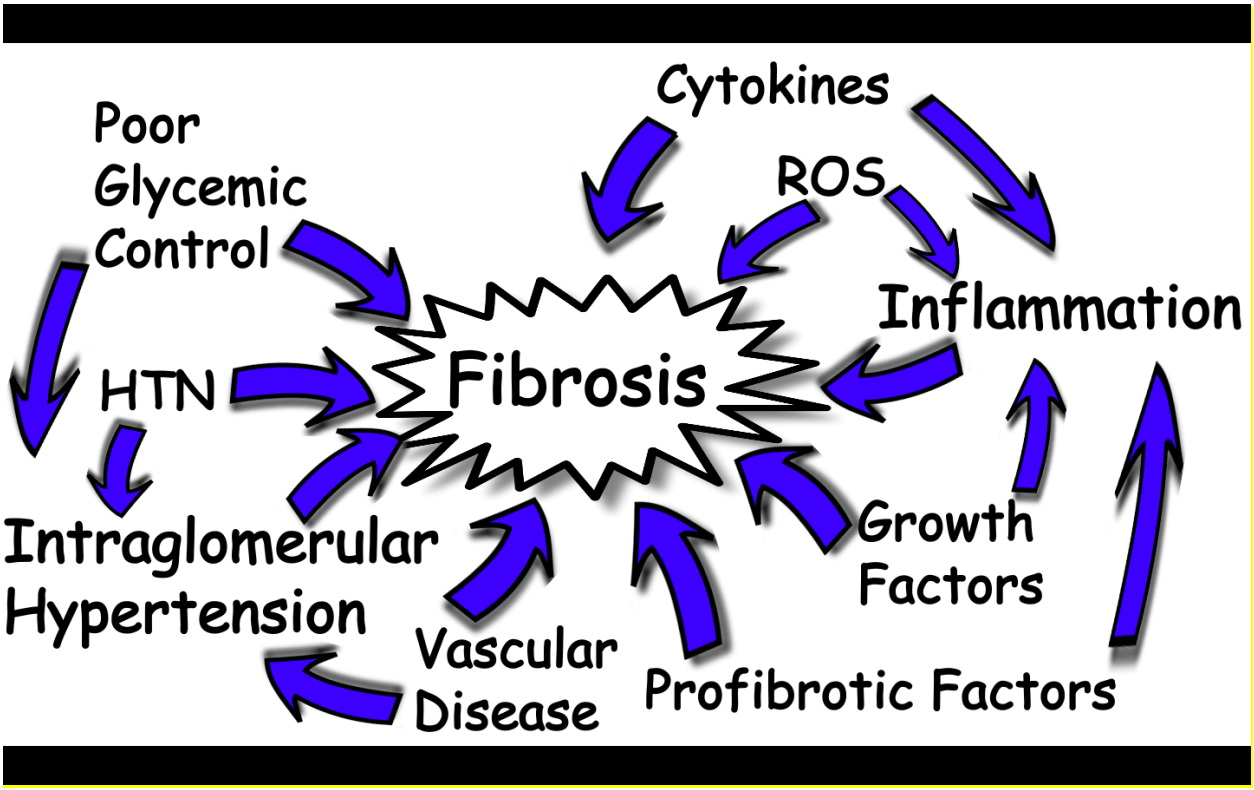

Figure 1. Contributing Factors in the development of Kidney disease. There are many processes that lead to Fibrosis within the kidney nephron. TNF-α, various interleukins and other cytokines, along with growth factors like TGF-α and TGF-β, and profibrotic factors like fibrin and fibrinogen all promote inflammation. Cytokines and reactive oxygen species (ROS) bring about further inflammation leading to glomerular, tubular, and mesangial matrix damage that results in glomerular and tubular fibrosis. Hypertension (HTN), vascular disease like atherosclerosis and arteriosclerosis, along with poor glycemic control as in diabetes lead to increased pressure within the glomerulus or intraglomerular hypertension which causes glomerular hyperfiltration leading to increased stress and ultimately fibrosis of the glomerulus and thus kidney disease.

Table 3: Anatomy of the Glomerulus

Nephron	Kidney's 'functional unit' consisting of a glomerulus, corresponding tubules, and blood supply.
Glomerulus	Highly regulated capillary bed through which blood is filtered. The resultant filtrate is taken up by the proximal tubule. Blood is fed into the glomerulus by the afferent arteriole and exits via the efferent arteriole. The glomerulus contains several capillary loops where the filtration occurs across the glomerular basement membrane (GBM).
Endothelial Cells	Vascular cell on the blood side (vascular side) of the GBM and capillary loop of the glomerulus. The endothelial cell is at particular risk for pathologic changes due to hemodynamic changes due to hypertension and atherosclerosis.
Tubules	The tubules of the nephron are continuous but are divided into multiple sections. Sections are designated by the proximity to the glomerulus, their distinct morphology, and their function. Sections include, in sequence, the proximal convoluted tubule, the loop of Henle, the distal convoluted tubule, and the collecting duct. At the distal end of the collecting duct the filtrate (urine) moves to the renal pelvis where it is emptied.
Mesangium	The mesangium, made up of mesangial cells, acts as the support structure for the nephron, serving as the scaffolding by which the glomerulus and tubules are able to communicate and function with one another. It plays a crucial role in establishing and maintaining the concentration gradient by which the filtrate is concentrated, allowing for solute and proteins to be filtered and reabsorbed as the filtrate travels along the tubules. Also plays a paramount role in assisting in the regulation of the glomerulus' pressures and arterioles. The mesangial cell is one of the culprit cells that can start the process leading to fibrosis (see Figure 1).
Podocyte	An epithelial cell that is part of the basement membrane of the glomerular vascular loops. Podocytes provide structure to the glomerular basement membrane (GBM) and contribute to the barrier limiting filtration of some substances across GBM. In diseases where there is abnormally excessive protein excretion in the urine, the podocytes are often affected and lose their typical structure. Like mesangial cells, podocytes are felt to be culprit cells that are early in the cascade of pathologic changes leading to glomerular fibrosis.

In patients with DKD continued elevations in blood pressure and blood glucose fuel overproduction and dysregulation of many inflammatory and fibrosis mediators (see Figure 1). This includes growth factors like transforming growth factor (TGF) – β, connective tissue growth factor, and fibroblast growth factor (FGF)-23. As mentioned earlier, the RAAS pathway is involved along with fibrinogen, tumor necrosis factor (TNF)- □ and endothelin (Barrera-Chimal and Jaisser 2020, Hirata, 2008, Mihai et al.,

2018). However, therapies specifically targeted at many of these factors or pathways other than the RAAS pathway, blood pressure reduction in general, and glucose metabolism itself have not made their way to clinical care. Moreover, in the patient with DKD, there are limited clinical measures on which a healthcare provider can base therapeutic decisions to preserve kidney function. These clinical measures are blood pressure, blood glucose control, and urine albumin excretion.

Angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor (AR) blockers

Angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) are a mainstay of therapy for all patients with CKD but particularly those with DKD (Crook, 2002, Rossing et al., 2018). RAAS inhibition slows progression of CKD and provides cardiovascular protection to patients with CKD (Mann et al., 2001, Rossing et al., 2018). Similarly, glycemic control has been long established to prevent development of and slow progression of CKD (Bilous, 2008), However, blood pressure control was felt to be relatively more important than glycemic control in preventing progression of DKD, and, until recently, it did not seem to make a difference as to which glycemic lowering agents were used when considering kidney function (Patel and Crook 2006). However, more recent data show that sodium-glucose-cotransporter 2 (SGLT2) inhibitors have beneficial effects beyond lowering the glucose levels. SGLT2 inhibitors likely as a class effect, prevent development of CKD in patients with DM, slow progression of DKD, and offer protection to CVD in patients with and without DM (Alicic et al., 2017, DeFronzo et al., 2021, McGuire et al., 2021, Zannad et al., 2020).

The impact of SGLT2 inhibitors beyond blood glucose control has led to hypotheses regarding alternative mechanisms, pathways, and factors involved in development of and progression of DKD. Poor glycemic control in DM results in glomerular hyperfiltration, an initial pathologic event in DKD. In conditions of high blood glucose, increased glucose reabsorption in the proximal convoluted tubule of the glomerulus occurs via SGLT2. This increase in glucose reabsorption sets off a cascade of events that result in increased intraglomerular pressure (Alicic, et al., 2017). The restoration of normal glomerular hemodynamics provided by SGLT2 inhibition likely dampens multiple proinflammatory and profibrotic factors responses set off by hyperglycemia (DeFronzo, et al., 2021). Hence, the unexpected additional benefits of a glucose lowering agent, beyond glucose lowering per se, has shed light into a pathologic pathway important in DKD.

The Mineralocorticoid Pathways and Receptors (MR)

To slow progression of DKD recent studies have taken a new look at the mineralocorticoid pathway and the mineralocorticoid receptor (MR) antagonists. The hemodynamic and metabolic derangements of DM lead to MR activation and alterations in inflammation and endothelial dysfunction (Patrycjusz, et al., 2018). Steroidal MR antagonists, like spironolactone and eplerenone, have been around for years but have numerous adverse effects and provide incomplete MR blockade. Newer nonsteroidal MR antagonists provide more complete MR blockade and fewer side effects as compared to steroidal MR antagonists. Recent studies have demonstrated that these nonsteroidal MR antagonists slow the progression of DKD and also provide CVD protection in patients with DM (Bakris et al., 2020, Buonafine, 2018, Kolodziejczyk et al., 2018). Nonsteroidal MR antagonists resulted in a significant reduction in albuminuria, one of the clinical measures that correlates with renal outcomes. The reduction in albuminuria was independent of effects on blood pressure, and like with SGLT2 inhibitors, is explained by hemodynamic effects within the glomerulus that blunt the proinflammatory and profibrotic downstream effects of MR activation. The SGLT2 inhibitors and nonsteroidal mineralocorticoid receptor antagonists have altered the landscape of treatment for DKD, and, if used broadly, should reduce the ethnic disparities seen in CKD/ESKD.

Advances in Reducing CVD Outcomes in Patients with CKD

Patients with Type 2 DM and CKD have 4 times the CVD mortality than patients without either of those conditions and 2 – 3 times the CVD mortality than patients with only one of those conditions (Afkarian, 2013). Moreover, patients with severe CKD (CKD

stage 4) have a two-fold increase in CVD mortality compared to patients with mild CKD (CKD stage 2) (Menon et al., 2008). Black individuals with CKD have worse outcomes than non-Hispanic Whites with CKD. Blacks are much more likely to progress to ESKD, which increases risk of CVD. In addition, Black individuals suffered 27% of deaths caused by ESKD in the US in 2018, a percentage more than twice their percentage in the US population (12%) (Benjamins, 2022, USRDS 2021).

Clearly, opportunities to lower CVD events and CVD mortality in patients with CKD will have a great impact. As mentioned above, blood pressure control with RAAS inhibition offers CVD protection in patients with CKD, particularly DKD. Unfortunately, glycemic control does not have the same impact on CVD in patients with DM as it does on DKD and other microvascular complications (Bilous, 2008). Moreover, the CVD protective effects of lipid lowering are not as impressive in patients with moderate to severe CKD as in the general population. The aforementioned observations of the beneficial effects of SGLT2 inhibitors and nonsteroidal MR antagonists on CVD events in patients with CKD add important therapeutic options in our battle against the primary cause of death in these patients. When combined with their impact on slowing of CKD progression, the beneficial effects on CVD make SGLT2 inhibitors and/or nonsteroidal MR antagonists important additions to the therapeutic cocktail for patients with DKD. These agents should also be considered in those patients without DM who have CKD.

Finally, patients with moderate to severe CKD or ESKD are more likely to not have the classic symptoms for coronary heart disease than the general population. Models for predicting cardiovascular risk in this population are being developed. Recent models have evaluated the use of newer cardiac and renal biomarkers. These investigators have found that inclusion of measures of glycemic control, inflammation, and heart and kidney injury in their models better predicted which patients with CKD would have a stroke or heart attack within 10 years (Bundy et al 2022). Improved predictive models for CVD in patients with CKD will hopefully address the excess deaths seen in AAs with ESKD (Benjamins et al., 2022).

Advances in Predicting Risk of CKD and Risk of CKD Progression

Despite the recent advances in therapeutics for CKD and the promising future on the horizon, prevention remains the mainstay of treatment. However, we continue to see high incidence and prevalence rates of DM, hypertension, and obesity – risk factors for CKD. As such we can expect the incidence and prevalence of CKD to continue increasing. It becomes even more important for us to make the diagnosis of CKD as early as possible, preferably when the disease is mild and interventions to slow or prevent progression of CKD are effective. Therefore, it is helpful, particularly to primary care providers, to have additional tools that predict which individuals are at increased risk to develop CKD or its risk factors. Such tools should promote enhanced screening and monitoring, and hopefully earlier diagnosis, of higher risk individuals.

There are several factors that alert us to the individual patients' risk for CKD. These include the presence of DM, hypertension, albuminuria, a family history of CKD, a history of acute kidney injury, and the presence of other conditions that may impact the kidney such as Lupus, HIV and viral hepatitis B and C. However, there is a significant number of individuals with CKD / ESKD where the renal diagnosis is not clear. A better understanding of the genetics of kidney disease will enhance our ability to identify those at higher risk for developing CKD and for having progression of their CKD.

It is estimated that approximately 10% of CKD in adults is hereditary (Devuyst et al 2014). While the two most common causes of CKD, DM and hypertension, are polygenic, there has been some progress in identifying genetic variants that affect the development and progression of CKD from these common causes as well as from none diabetes CKD (Groopman et al 2019, Groopman et al., 2020). Making a genetic diagnosis related to CKD can be significant for the patient and his/her family. A genetic diagnosis may enhance prognostic predictions, impact therapeutic decisions, affect family planning, enhance the earlier diagnosis of a

family member, and inform providers about the suitability of a potential kidney donor and potential for complications post-transplant (Groopman et al., 2018).

While a comprehensive review of the genetics of CKD is beyond the scope of this paper, we will introduce a couple of genetic variants that have clinical impact in CKD, particularly in AAs. The significant disparities in ESKD incidence and prevalence between AAs and non-Hispanic whites (four-fold higher in AAs), suggests some genetic factors may contribute to that higher risk (Kasembeli, et al., 2015). Chromosome 22 has been identified as a locus for genetic variants that may increase the risk of kidney disease in populations of African descent.

One of the genes found to be related to CKD is located at chromosome 22q12. The gene encoding non-muscle myosin heavy chain type II isoform A (MYH9) is expressed in podocytes in the kidney glomerulus and may be expressed in mesangial cells (see Table 3) (Kopp, et al., 2010). Mutations in the MYH9 gene have been associated with a 2-4 times increased risk of non-diabetic ESKD (Kao, et al., 2008). High risk alleles of the MYH9 gene are more frequently found in AAs than in Americans of European descent and have been associated with increased susceptibility to the development of Focal Segmental Glomerulosclerosis (FSGS), HIV Associated Nephropathy (HIVAN), and Hypertensive ESKD (Kopp, et al., 2008, Kopp, 2010). Moreover, protective alleles are more often found in European Americans, which suggests a potential explanation for the disproportionate nature of non-diabetic CKD in AAs (Kopp, et al., 2008, Kopp, 2010).

APOL1 is a serum factor that lyses trypanosomes, the parasite that causes African Sleeping-Sickness (Genovese, et al., 2010). Certain mutations in the APOL1 gene, also located on chromosome 22q12, are commonly found among certain African populations and in AAs. The mutations, referred to as G1 and G2, allow for the circulating factor to bypass the trypanosomes' resistant factors, thereby conferring survival advantage (Genovese, et al., 2010). Genetic variations in APOL1 are linked to increased risk of CKD in African Americans (Genovese, et al., 2010, Parsa, et al., 2013). Among African Americans 30% have at least one risk allele and 17% have 2 risk alleles (Parsa et al., 2013). High risk APOL1 variations explain approximately 70% of the excess risk for FSGS and ESKD in AAs (Genovese, et al., 2010). The impact of APOL1 in CKD due to hypertension and DKD is varied. In patients with CKD due to hypertensin, APOL1 variants confer higher risk for progression to ESKD. In patients with diabetes mellitus, APOL1 is not associated with the development of DKD. However, in those patients with established DKD, APOL1 is a risk factor for progression of DKD to worse stages of CKD/ESKD (Genovese, et al. 2010, Parsa et al., 2013, Freedman 2010).

Unlike the strong associations of APOL1 and MYH9 with certain forms of CKD in individuals of African descent, we do not have genetic variations that are as strongly linked with the development and progression of DKD. There are over 500 single nucleotide polymorphisms (SNPs) associated with DM. The polygenic risk score (PRS) is an approach to determine genetic risk for a disease or condition by combining the effects of multiple genetic variants across the genome. Among individuals with DM, those with highest decile of PRS have much greater likelihood of developing the microvascular complications of DM, retinopathy (OR 1.59), neuropathy (OR 1.21), and nephropathy (OR 1.16), than those with PRS in lowest decile (Vujkovic, et al., 2020, Khera, et al., 2018). Prediction of risk may be improved when clinical factors are included in the computation. This approach will facilitate the identification of a group that requires more aggressive screening, monitoring and intervention.

Lastly, urine proteomics is being utilized to enhance prediction of which patients with DKD are likely to have progression of their kidney disease. CKD 273 is analysis of urine via capillary electrophoresis coupled with mass spectroscopy (CE-MS) that detects 273 peptides in the urine. Many of the peptides are markers of kidney fibrosis. In a study of individuals with DM without albuminuria, those individuals with high risk profile on CKD 273 were 3 times more likely to develop microalbuminuria, the earliest clinical sign of DKD, than those with the low risk profile (Tofte et al., 2020).

SUMMARY AND CONCLUSIONS

CKD is common and immensely costly in the quality of human life, loss of life and financial costs. Unfortunately, the majority of Americans with CKD are unaware of their diagnosis as it is typically asymptomatic in earlier stages. For DKD, the most common cause of CKD, there are new therapeutic agents that slow the progression of DKD, delay the onset of DKD, and reduce CVD events in patients with DKD and without DM. These pharmacologic advances combined with advances in predicting those at most risk for CKD (and DKD) via genetic testing mark a significant point in our ability to impact CKD. The medical community has a great opportunity to lower the incidence and prevalence of CKD. These advances are the most impactful since the realization that RAAS inhibition slowed progression of DKD. The health inequities in CKD have to be considered as we implement these new advances. Ethnic minorities, the poor and those with lower educational attainment suffer the biggest burden of CKD and are at greatest risk for not receiving the current standards in care, much less advances in care. The medical and scientific community must advocate for the broad dissemination of these advances so that the disparities in CKD are eliminated, in keeping with the vision of Healthy People 2030, which has a specific initiative to reduce CKD and its complications.

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Transition from Hypertension to Heart Failure and Impact of Racial Disparities

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ABSTRACT

Hypertension is the major attributable risk factor for cardiovascular diseases (CVD) including heart failure (HF). As blood pressure increases, the risk of HF increases concomitantly. The incidence of HF continues to increase as people are living longer with comorbid diseases, and HF is a leading cause of morbidity and mortality in the United States. Black adults are disproportionately affected by hypertension and HF and develop CVD at younger ages compared to White adults. There are several factors which may contribute to these disparities including metabolic factors and sociodemographic factors. Treatment of hypertension can reduce the risk for HF. Lifestyle modification can be effective at reducing the risk of both hypertension and subsequent HF. Anti-hypertensive medications are available which are effective, safe, and cost-effective. Despite wide recognition that hypertension is a strong risk factor for HF, effective treatments for hypertension, and guideline recommendations for better blood pressure control, hypertension control rates remain poor in the United States. In this paper, we discuss the relationships between hypertension and HF, the impact of racial disparities on hypertension and HF, and prevention and treatment strategies.

Key words: Anti-hypertensive-medications, Blood-Pressure (BP), Ejection- Fraction (EF), Heart- Failure, Hypertension, Hypertrophy, Racial- Disparities

INTRODUCTION

Hypertension is a major risk factor for cardiovascular diseases (CVD) worldwide. Normal blood pressure (BP) in adults is defined as a systolic BP (SBP) < 120 mmHg and a diastolic BP (DBP) < 80 mmHg (Whelton, 2018). Based on the most recent American College of Cardiology (ACC)/American Heart Association (AHA) BP Guidelines, hypertension is defined as SBP > 130 mmHg or DBP > 80 mmHg (Whelton, 2018). Based on population studies from 90 countries around the world, 31% of adults (1.39 billion people) had hypertension defined as a SBP ≥ 140 mmHg or DBP ≥ 90 mmHg (Mills, 2016). Estimates in the United States using the National Health and Nutrition Examination Survey (NHANES) data, demonstrated over 103 million adults (46%) had hypertension (Centers for Disease Control and Prevention, 2021). The most recent NHANES Survey (2017-2018) data using a cut-point of 130/80 mmHg was consistent with previous reports at near 45% prevalence (Rana, 2020). Using pooled data from large representative cohort studies in the United States, the cumulative lifetime risk for hypertension from ages 20-85 years of age was 85%.

There are several non-modifiable risk factors for hypertension such as older age, male sex, race, and genetic factors. However, many risk factors for hypertension such as obesity, reduced physical activity, obstructive sleep apnea, diabetes, smoking, and high sodium intake are modifiable (Whelton, 2018). Other factors including stress and low socioeconomic status are strongly linked with increases in BP. Age-related increases in BP occur in almost every population. Increased weight and adiposity are strongly associated with hypertension and it is estimated that up to 75% of hypertension in humans is due to overweight or obesity (Hall, 2015). These cardiometabolic factors often occur together

and may act synergistically to increase BP or increase the risk for CVD.

Heart failure (HF) is a clinical syndrome manifested by characteristic signs and symptoms including dyspnea, edema, and cardiac dysfunction. Heart failure has been classified into subtypes based on left ventricular (LV) ejection fraction (EF): 1) HF with reduced ejection fraction (HFrEF; EF ≤40%), 2) HF with midrange ejection fraction (HFmrEF; EF 41%-49%), and 3) HF with preserved ejection fraction (HFpEF; EF ≥50%). Although the symptoms of HF are similar across HF subtypes, the classification of HF is important because there are different treatment strategies based on the subtype of HF a patient has (Heidenreich, 2022). Furthermore, HF is classified into different New York Heart Association Classes (NYHA) I-IV based on clinical severity and stages from A (at risk of HF) to D (advanced HF) (Heidenreich, 2022) (Figure 1). Regardless of the differences in treatment strategies, hypertension is a major risk factor for all types of HF and thus BP control should be targeted for prevention of HF.

As people are living longer, chronic diseases like HF are becoming more prevalent. Recent estimates suggest that more than 6 million people in the United States have HF (Benjamin, 2018). Heart failure is a major cause of morbidity and mortality. The ARIC study showed 30-day, 1-year, and 5-year case fatality rates after hospitalization for HF were 10.4%, 22%, and 42.3%, respectively (Loehr, 2008). Although there are differences in pathophysiology and available treatments, 5-year mortality rates for HFrEF and HFpEF are similar (75.3% vs 75.7%, respectively) (Shah, 2017). Besides hypertension, other risk factors for HF include age, race, diabetes, smoking, atrial fibrillation, coronary heart disease/myocardial ischemia, valvular heart disease, genetic conditions, and obesity (Virani, 2021).

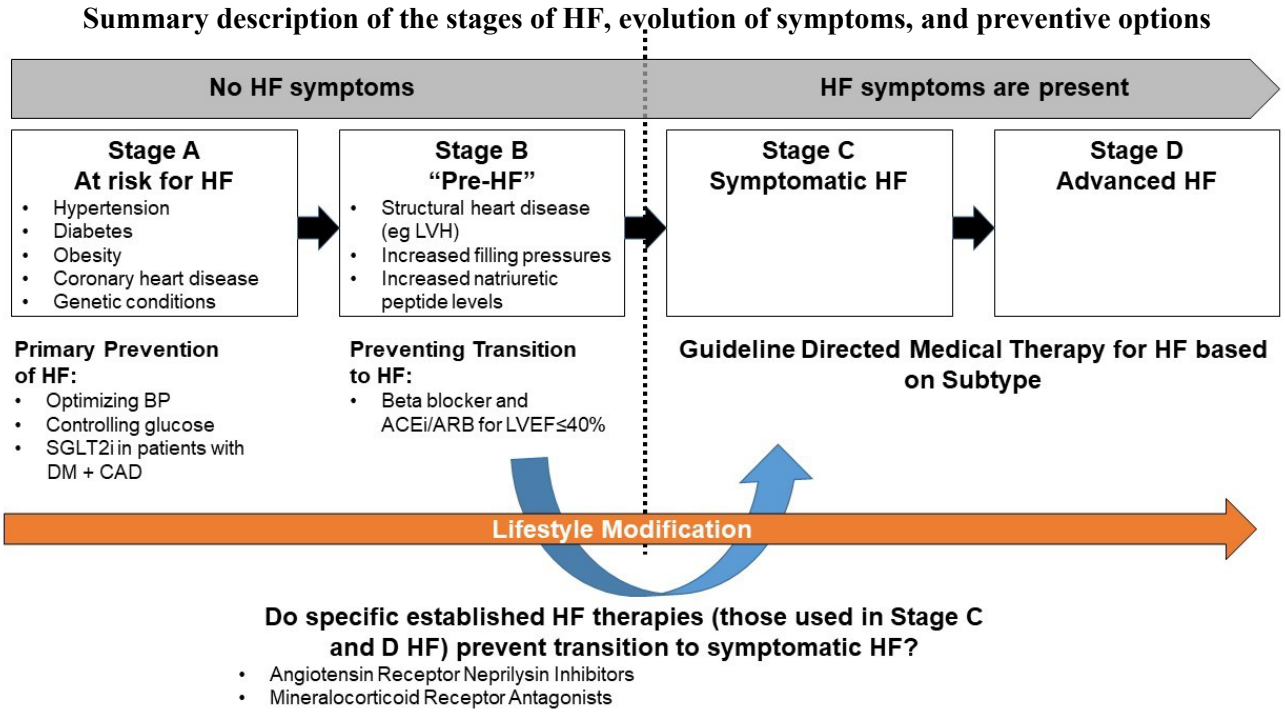

Figure 1. The stages of heart failure and evolution of symptoms. Some strategies for preventing progression of early stage heart failure (stages A and B) to symptomatic heart failure are already recommended. However, it remains unclear if initiation of certain medications for prevalent heart failure (stages C and D) in these early stages will prevent this progression.

HF= heart failure; BP= blood pressure; LVH= left ventricular hypertrophy; DM= diabetes mellitus; CAD= coronary artery disease; ACEi= Angiotensin converting enzyme inhibitor; ARB= Angiotensin receptor blocker;

Racial Disparities in Hypertension and Heart Failure

The prevalence of hypertension and HF differ based on race and ethnicity. Black Americans have a higher prevalence of hypertension than other racial/ethnic groups. Based on the NHANES Survey data (2015-2018), the prevalence of hypertension was 56.2% in non-Hispanic Black adults compared to 48% in non-Hispanic White adults in the United States (Ostchega, 2020). Black men had the highest rates of hypertension compared to White and Hispanic men, and Black women had disproportionately higher prevalence of hypertension compared to women of other racial/ethnic groups (Table 1) (Ostchega, 2020). Black individuals also develop hypertension at earlier ages compared to White individuals, they tend to have higher average BP, and they are more likely to have refractory hypertension compared to White individuals (Calhoun, 2014; Lackland, 2014). The explanatory factors related to these disparities are not clear but may include variability in sodium intake/retention, salt sensitivity, higher burden of adiposity, and socioeconomic factors which disproportionately affect Black individuals (Lackland, 2014). Some of these socioeconomic factors such as poverty, food insecurity, and reduced access to quality health care may interact with other genetic or environmental factors to increase to risk of hypertension and related CVD.

Black adults are more sensitive to increases in cardiac afterload and are thus more likely to exhibit LV remodeling earlier than other races/ethnicities (Fernandes-Silva, 2017). Furthermore, Black adults have an increased risk of LVH and approximately a 2-fold greater risk of concentric remodeling compared to White adults (Kizer, 2004). Additionally, Black adults already have an increased predisposition to develop HFpEF compared to HFrEF (Gupta, 2013). This predisposition paired with concentric remodeling patterns favor the development of HFpEF in Black adults with hypertension.

Heart failure disproportionately affects Black adults. Black adults have the greatest risk for incident HF compared to other ethnicities and almost a 2-fold increased incidence rate of HF compared to White adults (4.6 vs 2.4 per 1000 person-years, respectively) (Bahrami, 2008). Furthermore, Black adults have a greater risk of death from HF compared to White adults with a 51.8% vs 41.2% age-adjusted 5year case fatality, respectively in men. These findings remained consistent for women as well (Loehr, 2008).

Despite the well-known higher risk for hypertension and HF risk in Black individuals, they are less likely to achieve BP control while being treated for hypertension (Sharma, 2014). This is despite findings from robust randomized clinical trials such as SPRINT demonstrating intensive BP control was associated with similar benefits and risks in all racial groups. However, it is worth noting that Black participants in Systolic Blood Pressure Intervention Trial (SPRINT) did require about 0.3 greater anti-hypertensive medications to control BP than other racial groups (Still, 2017). Given the higher rates of both hypertension and HF in Black adults, more emphasis on intensive BP control in this higher risk population is warranted.

The Role of Hypertension in Development of Heart Failure

Hypertension is one of the greatest attributable risk factors for HF. The Framingham Heart Study estimated 39% and 59% population attributable risk ratios in men and women, respectively (Levy, 1996). There is a continuous positive association between increasing BP and risk of developing HF. In observational studies, the risk of incident HF increased across BP categories with a hazard ratio (HR) of 1.63 in people with prehypertension (SBP 120-139 mmHg), HR 2.21 in those with stage 1 hypertension (SBP 140-159 mmHg), and 2.60 in those with stage 2 hypertension (≥ 160 mmHg) (Butler, 2011).

Elevated BP leads to increased peripheral vascular resistance and afterload. This increase in pressure can lead to compensatory concentric LV wall thickening to prevent decompensation in the setting of high intracardiac pressures (Oh, 2020). Sustained increases in BP or afterload can result in LV

hypertrophy (LVH), specifically concentric LVH. Left ventricular diastolic dysfunction is considered one of the first signs of hypertensive heart disease and there is evidence that diastolic dysfunction begins in patients with hypertension even prior to the onset of concentric LVH (Pavlopoulos, 2008; Slama, 2002). While concentric LVH remains a major contributor to the development of diastolic dysfunction in patients with hypertension, other mechanisms may play a role as well. These include intrinsic myocyte impairment, interstitial myocardial and perivascular fibrillar collagen deposition, and myocardial ischemia (even in the absence of signs of coronary artery lesions) (Slama, 2002).

Chronic hypertension involves excess pressure and volume. Therefore, in addition to concentric hypertrophy due to increased pressure, eccentric hypertrophy can also develop as a result of excess volume (Oh, 2020). Different demographic characteristics favor different remodeling patterns. Female gender, Black racial/ethnic identity, and older age are associated with concentric hypertrophy (Kizer, 2004; Krumholz, 1993; Oh, 2020). Male gender, obesity, and coronary artery disease predispose have been associated with eccentric hypertrophy (Krumholz, 1993).

Stage B HF or "pre-HF" is defined as evidence of: 1) structural heart disease, 2) evidence of increased cardiac filling pressures, or 3) HF risk factors with increased cardiac biomarkers (Heidenreich, 2022) in patients without signs or symptoms of HF. Therefore, patients with LV diastolic dysfunction or concentric LVH from hypertension are classified as having stage B HF or "pre-HF." Once they develop signs or symptoms of HF such as lower extremity edema, dyspnea, or orthopnea they would transition to at least stage C HF (Figure 1).

The Multi-Ethnic Study of Atherosclerosis (MESA) demonstrated that increased LV mass and increased LV volume both are associated with a 40% and 30% increased risk of HF, respectively (Bluemke, 2008). While increases in wall thickness, LV dimensions, and cardiac mass have all been associated with increased risk for HF, there is some evidence that different remodeling patterns may be associated with differential risk of HF. Investigators from the Framingham Heart Study showed that the highest risk for incident HFrEF (HR 2.23) was observed in participants with eccentric hypertrophy. However, participants with concentric LVH were more prone to HFpEF (HR 1.66) (Velagaleti, 2014). Regardless, increases in LV mass and adverse cardiac remodeling are predictors of incident HF and patients with these findings should be targeted for prevention and treatment strategies.

Blood Pressure Control in the Prevention of Heart Failure

There is robust experimental data that increased BP in animal models of hypertension leads to HF, observational human data associating increased BP and hypertension with increased risk of HF, and clinical trial data demonstrating reduced risk of HF events in patients with BP treatment. A large meta-analysis of 123 studies including >613,000 participants estimated a 28% reduced risk of HF for every 10 mmHg reduction in SBP (Ettehad, Emdin et al. 2016). The Systolic Hypertension in the Elderly Program (SHEP) evaluated the occurrence of HF in patients at least 60 years of age with isolated systolic hypertension and compared those treated with placebo with participants treated to a goal BP < 160 mmHg using stepped care with chlorthalidone and atenolol. Active anti-hypertensive treatment was associated with 49% relative risk reduction in fatal and nonfatal HF (Kostis, 1997). The more recent landmark SPRINT trial compared intensive BP control to a target SBP of 120 mmHg to 140 mmHg (standard) and observed a significantly lower rate of CVD events and death in those treated in the intensive BP control arm (Group, 2015). Subsequently, the ACC/AHA BP guidelines lowered recommended BP treatment target to a goal of < 130/80 mmHg (Whelton, 2018). After 3.3 years of follow up in SPRINT, intensive BP reduction was associated with 37% lower risk for incident HF compared to standard BP control (Group, 2015).

Regression of LVH has been associated with reduced risk of incident HF in patients with hypertension (Okin, 2007). In SPRINT, intensive BP control was associated with 46% lower risk of developing electrocardiographic LVH. Also, participants in the intensive BP control arm were 66% more likely to

have LVH regression (Soliman, 2017). Therefore, BP control not only reduces the risk for developing incident HF, it can prevent the development of HF precursors such as LVH and lead to regression of LVH.

Blood Pressure Control and Treatment of Hypertension

Blood pressure control and treatment of hypertension can be achieved through several strategies including lifestyle modifications, pharmacologic treatments, or surgical or device-based treatments. Lifestyle modifications including a healthy diet, increased physical activity, and reduced sedentariness should be recommended for all individuals with increased BP and those at increased risk of HF. Adherence to a heart-healthy diet such as the Mediterranean diet or DASH (dietary approaches to stop hypertension) diet are associated with lower BP (SBP reduction of 5.5 mmHg and 11.5 mmHg, respectively) and a decreased risk for incident HF (Jennings, 2019; Levitan, 2009a, 2009b; Sacks, 2001; Tektonidis, 2016). Furthermore, sodium restriction of < 2 g/day is associated with BP reduction and while the role of sodium intake in treatment of HF is controversial, it is recommended by the ACC/AHA in the management of stage C HF (Heidenreich, 2022; Mozaffarian, 2014; O'Donnell, 2020). Reducing alcohol consumption should be encouraged in patients who drink more than 2 drinks per day. This is associated with BP reduction in a dose dependent manner and patients who drink less ≤ 2 drinks/day also have a lower risk of HF (Klatsky, 2005; Roerecke, 2017). Increased physical activity via endurance, dynamic resistance, isometric resistance training lowers SBP and DBP (Cornelissen, 2013). Moreover, increasing physical activity as well as decreasing sedentary time are interventions to prevent HF (Young, 2014). Good sleep quality and managing sleep apnea are associated with prevention of hypertension and HF. An apnea-hypopnea index of 15 (suggestive of mild-moderate sleep apnea) is associated with increases in SBP by 3.6 mmHg and DBP by 1.8 mmHg (Young, 1997). Sleep apnea is associated with a 140% increased risk of HF (Jean-Louis, 2008). Taken together, the impact of a healthy lifestyle is significant and is recommended at all stages of hypertension (particularly patients with elevated BP i.e. 120-129/<80 mmHg) to prevent HF and is also recommended in the management of prevalent HF (Whelton, 2018).

Increased adiposity and excess weight are strongly associated with increased BP, hypertension, and HF. Weight loss has a significant impact on reduction in BP with an estimated 1 mmHg reduction in SBP for every 1 kg reduction in weight (Neter, 2003). Unfortunately, weight loss is difficult to sustain over the long-term and weight regain is common. Therefore, other weight loss strategies including weight loss pharmacotherapy or bariatric surgery can be considered for long-term weight reduction and BP control in obese patients with hypertension. Currently, there are 5 medications approved by the Food and Drug Administration for obesity (Hall, 2021). Most of these have modest effects to reduce BP. Recently, the glucagon-like peptide-1 receptor agonist, semaglutide was shown to significantly reduce weight by about 15% in obese individuals and this was associated with about a 6 mmHg reduction in SBP (Wilding, 2021). While medications are approved to treat obesity that may have positive impact on BP control, they are rarely prescribed (Zhang, 2016). Bariatric surgery is a very effective way to reduce weight in obese individuals. The GATEWAY (Gastric Bypass to Treat Obese Patients with Steady Hypertension) trial demonstrated that 84% of obese patients with hypertension were able to achieve the primary outcome of a ≥ 30% reduction in the number of anti-hypertensive medications and there was a 46% remission of hypertension based on ambulatory BP monitoring (Schiavon, 2018). Bariatric surgery also has beneficial effects to reduce LV mass in obese people and has been associated with a 46% reduction in incident HF events in observational studies (Sundstrom, 2017). The role of weight loss in management of prevalent HF is controversial due to the “obesity paradox” (Powell-Wiley, 2021); however, abundant evidence supports weight loss to prevent and treat hypertension which is the major risk factor for HF.

There are many effective anti-hypertensive medications which are available to treat hypertension. Anti-hypertensive medications are indicated in stage 1 hypertension (130-139/80-89 mmHg) with an atherosclerotic cardiovascular disease (ASCVD) risk

of > 10%. If ASCVD risk < 10%, then lifestyle intervention should be attempted prior to initiating medications. Anti-hypertensive medications are also indicated for stage 2 hypertension (> 140/90 mmHg). Thiazide/thiazide type diuretics, angiotensin converting enzyme inhibitors (ACEi), angiotensin receptor blockers (ARB), or calcium channel blockers (CCB) are considered first-line medications for hypertension. Secondary agents include beta blockers and mineralocorticoid receptor antagonists (Whelton, 2018).

Based on robust data from randomized clinical trials, there are guideline recommendations for treatment of prevalent HF. There are also guideline recommendations for specific medical therapies, many of which are anti-hypertensive medications, for treatment of HF subtypes (HFrEF and HFpEF). However, these guidelines do not distinguish if certain anti-hypertensive medications should be utilized in patients at increased risk of HF (stage A HF) or pre-HF (stage B HF) (Heidenreich, 2022). Renin angiotensin aldosterone inhibition via ACEi, ARBs, mineralocorticoid receptor antagonists (MRA), and angiotensin neprilysin inhibitors (ARNI) are associated with favorable LV remodeling, including increases in LVEF and reductions in LV mass index (Ferrario, 2016; Li, 2013; Schmieder, 1996). Furthermore, administration of MRA in addition to ACEi lead to even greater improvements in LVEF and LV end diastolic index (Hayashi, 2003). While ARNI therapy is not indicated for treating hypertension without stage C or D HF, the PROVE-HF (Prospective Study of Biomarkers, Symptom Improvement, and Ventricular Remodeling During Sacubitril/Valsartan Therapy for Heart Failure) study demonstrated significant LV remodeling benefits with significant reductions in end-systolic and -diastolic LV volumes as well as improvements in LVEF in participants receiving ARNI treatment (Januzzi, 2019). In salt-sensitive individuals with hypertension, ARNI treatment reduced SBP and natriuretic peptide levels more than ARB treatment and in older patients with hypertension ARNI treatment had greater effects to reduce central aortic pressure (Wang, 2017; Williams, 2017). Since these populations are at higher risk for HF and ARNI therapy seems to have greater beneficial effects on predictors of HF, it is possible that treating patients with hypertension who may be at higher risk for HF with ARNI could be a strategy to reduce incident HF (Figure 1). However, there are no randomized clinical trials assessing this question and further investigation is warranted.

Several anti-hypertensive medications have been associated with reduced risk for incident HF. While lowering BP is associated with lower risk of developing HF in patients with hypertension, some classes of anti-hypertensive medications have demonstrated greater risk reduction for developing HF. In the Anti-hypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT), treatment with the thiazide diuretic chlorthalidone was associated with a 35% lower risk of hospitalized HF compared with the CCB amlodipine (ALLHAT Officers, 2002). In the Heart Outcomes Prevention Evaluation (HOPE) study in patient at high risk of CVD, the ACEi ramipril was associated with a significantly lower risk of incident HF compared to placebo (Arnold, 2003). While CCB are effective at lowering BP, a recent Cochran Database Review demonstrated an increased risk for incident HF compared with ACEi and ARB (Zhu, 2021). Beta blockers generally have weaker BP lower effects than other classes of anti-hypertensive medications. In a meta-analysis of beta blocker treatment compared to other anti-hypertensive agents, beta blockers had no additional benefit compared to other anti-hypertensive agents in the prevention of HF (Bangalore, 2008). However, they are one of the most beneficial therapies in patients with prevalent HFrEF. Given the favorable effects of MRA to effectively reduce BP, reduce LVH, and positive impact on markers of myocardial fibrosis, there are ongoing clinical trials evaluating their use in patients at risk of developing HF (stages A and B HF).

Unfortunately, most clinical trials assessing anti-hypertensive medications in prevention of HF did not include many Black participants and it remains unclear if certain therapies may be more beneficial in Black individuals at higher risk for HF. Thus, adequately powered clinical studies inclusive of more diverse populations are warranted to determine if certain anti-hypertensive treatment strategies are more beneficial in certain racial/ethnic groups.

Blood Pressure Control Rates Remain Poor

Despite wide recognition that hypertension is a major risk factor for HF, BP control rates remain poor. Based on data from the NHANES study (2015-2018), the majority of adults within the United States with hypertension are uncontrolled. Among patients with hypertension who are recommended pharmacologic intervention, 73.9% remain uncontrolled (Centers for Disease Control and Prevention, 2021). This is concerning given we have many effective, safe, and cheap anti-hypertensive medications which are widely available in the United States. Several factors likely contribute to suboptimal BP control including patient non-adherence, limited access to healthcare, and clinician inertia. Many of these factors disproportionately affect Black individuals and BP control is less likely in Black compared to White adults (Muntner, 2020).

Low adherence is a major factor for uncontrolled hypertension. Many factors including complex medical regimens, high pill burden, and issues with side effects play a role in patient non-adherence for a predominantly asymptomatic disease. Fortunately, most anti-hypertensive medications are generic and are low cost and the side effect profiles are generally good. Patient awareness of having hypertension and insight into the risk of HF associated with untreated hypertension also play a role. Patients who have a usual healthcare facility and those who attended a healthcare facility in the past year are more likely to be aware of their hypertension (Muntner, 2020). Thus, access to care is an important factor for controlling BP and reducing risk of HF, and Black individuals may have less access to quality healthcare.

In addition to patient non-adherence to anti-hypertensive medical regimen, non-adherence to lifestyle modification can contribute in HF risk in patients with hypertension. Large population studies have demonstrated low levels of ideal lifestyle factors including inadequate physical activity and high body mass index in Black adults.

Although there are several patient-related factors that affect BP control and HF risk, there are also clinician-related factors which contribute. In a large ambulatory study of nearly 42 million primary care visits among adults with hypertension in the United States with a BP ≥ 140/90 mmHg, only 17% had anti-hypertensive medication intensification with a new medication. Many factors contribute to clinician inertia for BP control and these same factors play a role in inertia to initiate or up titrate guideline directed medical therapies for prevalent HF (Mu, 2016). These factors include limited time (clinicians taking care of complex patients with multiple medical problems), concern about side effects of medications, uncertainty of contemporary guidelines and treatment goals, and uncertainty of accuracy of BP measures. Thiazide diuretic and MRA use are effective in the treatment of resistant hypertension (Whelton, 2018). However, few Black patients with apparent treatment-resistant hypertension received a thiazide diuretic (6%) or a mineralocorticoid receptor antagonist (10%) (Langford, 2020).

The healthcare system is strained, primary care providers are increasingly overwhelmed, and patient- and provider-level factors continue to adversely impact BP control rates. To prevent the risk of hypertensive heart diseases such as HF, strategies incorporating patients into the decision-making process and treatment plan, integration of team-based care across different levels of healthcare providers, and models of care delivery outside of the traditional in-person physician office visit (e.g. remote patient monitoring) may provide opportunities for more optimal BP control to reduce the risk for HF.

CONCLUSIONS

Hypertension is *the* major risk factor for HF. With an increasing aging population, the burden of HF will continue to increase so targeting HF prevention strategies is of utmost importance. Controlling BP can reduce the risk of HF precursors such as abnormal cardiac remodeling and can also reduce the risk for developing incident HF. Some classes of anti-hypertensive medications may have greater effects to protect against developing HF. Further investigation of specific anti-hypertensive treatment strategies in patients with early stage HF (stages A or B) are warranted to determine the optimal preventative strategies in patients with hypertension. Black adults have higher rates of hypertension and HF, worse BP control, and worse HF outcomes compared with

White adults. Although overrepresented in disease prevalence, Black adults are underrepresented in clinical studies of these diseases so optimal treatment strategies for this higher-risk population are not clear. Although hypertension is widely recognized as a major risk factor for HF, and we have safe, cheap and effective anti-hypertensive medications, BP control rates remain poor. Ultimately, strategies aimed at addressing patient- and provider-level factors associated with suboptimal BP control will be necessary to attenuate the increasing risk of subsequent HF.

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Vaccine Development and Hesitancy

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ABSTRACT

Infectious diseases have shaped human and societal development. Infectious diseases have influenced everything from trade routes to animal health, and survival of established cultures. Devastating consequences have resulted from The Black Death, diphtheria, polio, and smallpox. The SARS-CoV-2 pandemic reinforced the observation that in addition to human health, infectious diseases have affected the global economy and climate. The most effective development in medical history has been vaccines. Vaccines conquered smallpox the first human infectious disease eradicated worldwide. Historically, vaccines were developed by inactivating a pathogen for immunization or identifying a component of a pathogen that would elicit a protective immune response following injection. With advances in molecular approaches, such as whole-genome sequencing, have facilitated vaccine development. The result is that there are now novel vaccines in development against cancer, contraception, drug abuse, as well as emerging infectious agents. While vaccines have had a positive impact, there has always been some level of hesitancy toward vaccines.

KEY WORDS: Anti-Vaccine, Disease Prevention, mRNA Vaccines, Vaccines, Vaccine Development, Vaccine Hesitancy

INTRODUCTION

Precision medicine strives to prevent diseases rather than intervene in an ongoing disease. Vaccines are among the most effective disease prevention approach in modern medicine. The smallpox vaccine is so effective at preventing infection with the virus that smallpox has been eradicated from the world. However, some vaccines alter disease outcome rather than prevent infection. While the COVID-19 vaccine does not prevent infection in all immunized individuals, it significantly reduces mortality and the need for hospitalization (Fiolet, 2022;). Other vaccines do not eliminate the infecting microbe but are able to neutralize bacterial toxins that can cause harm and even death to infected individuals. For example, immunization with attenuated bacterial toxins, known as toxoids, can prevent tetanus and diphtheria (Delany, 2014;). Following improved virus cultivation techniques, vaccines were developed against influenza and yellow fever. Subsequently, with the rapidly evolving field of molecular biology including recombinant DNA technology there have been many advances in vaccine development. Joined with other precision medicine approaches, vaccines have greatly improved human and animal health.

Vaccine Concepts

Vaccines are attenuated pathogens or a component of a pathogen that when used to immunize an individual will elicit a protective immune response. Thus, vaccination is the process of injecting susceptible individuals with a biological substance designed to produce protection against a given disease. This is a way of producing adaptive immunity in a susceptible host. Adaptive or acquired immunity allows an individual's immune system to distinguish self

from non-self. Acquired immunity rapidly recognizes an invading pathogen and neutralizes any harmful effects from the pathogen. While vaccination is an artificial means of eliciting specific acquired immunity, vaccines produce a more robust protective immune response compared to natural infection.

Vaccines are meant to protect individuals who are at risk of an infectious disease. Young children, the elderly, immune-compromised individuals, and people living in disease endemic areas are at risk from certain infectious diseases. Vaccination offers an effective means to control and perhaps eliminate disease among an at-risk population. Vaccines can be live or killed microbes, a component of a pathogen, a pathogen component conjugated to a carrier, or recombinant nucleic acid from a pathogen. A live vaccine can replicate in the host and may only require a single dose to elicit protection. The other types of vaccines may require multiple injections (boosters) to elicit protection.

Another vaccine concept is a correlate of protection. Correlates of protection asses the immune response to a vaccine within the immunized at-risk population (Callegaro and Tibaldi, 2019;). A correlate of protection can be a measure of the antibody response to the vaccine. Thus, after vaccination if an individual has a certain level of antibody against the vaccine, there is a strong correlation that they will be protected from infection. This provides a measure of efficacy of the vaccine.

Why we forget

Overall, vaccines are highly effective. A potential side effect of vaccines is that we forget about the disease we are protected against. For example, prior to the availability of the pertussis vaccine, whopping cough was a major killer of young children in the United States. Yet today almost none of us have personally heard the agonizing gasp for air of a young child with whopping cough. The effectiveness of the pertussis vaccine is the reason we have forgotten about this disease and some people feel their child no longer needs this vaccine. However, in some areas of the country, whooping is resurging among those children who have not been vaccinated.

Vaccine development

Vaccines play an important role in human and animal health by preventing infection and transmission of disease. Vaccine development began by immunizing people with live attenuated viruses. This was followed by using killed microbes for immunization. Subsequently, subunit vaccines became the standard approach to vaccine development. The latter approach is considered a reductionist approach. Instead of using an entire microbe for immunization, a subunit or component of the microbe that could elicit protection against the infection was identified and used as a vaccine. These individual components from a microbe are known as antigens. An antigen can stimulate the immune system to identify an invading pathogen. Subunit vaccines are considered safer since they elicit a specific response rather than immune responses to the entire microbe. While safer, subunit vaccines require boosting with multiple injections over time.

There are challenges in developing subunit vaccines. For example, *Streptococcus pneumoniae* (pneumococcus) is a leading cause of death throughout the world of children under five years of age. It has been known for over 100 years that the capsular polysaccharide that surrounds the pneumococcus is an important virulence factor that has the potential to elicit a protective immune response (Avery and Dubos, 1931; Grabenstein and Klugman, 2012;). Unfortunately, there are over 100 pneumococcal serotypes. This means that a purified capsular polysaccharide from one serotype can elicit protection against that serotype but not the other 99 serotypes. In the 1970s a vaccine composed of purified polysaccharide from the 13 pneumococcal serotypes commonly associated with adult infections was developed (Austrian, 1979;). Subsequently, an additional 10 purified capsule serotypes were added to the vaccine to offer broader coverage. An additional problem with this 23-valent vaccine is that children less than two years of age, who are a major risk group, fail to make an immune response against purified

polysaccharides. To overcome this problem, the purified polysaccharides have been conjugated to a protein carrier (Figure 1). While this allows young children to mount a protective immune response, there is still the challenge of protecting against the other nearly 80 serotypes that are not covered.

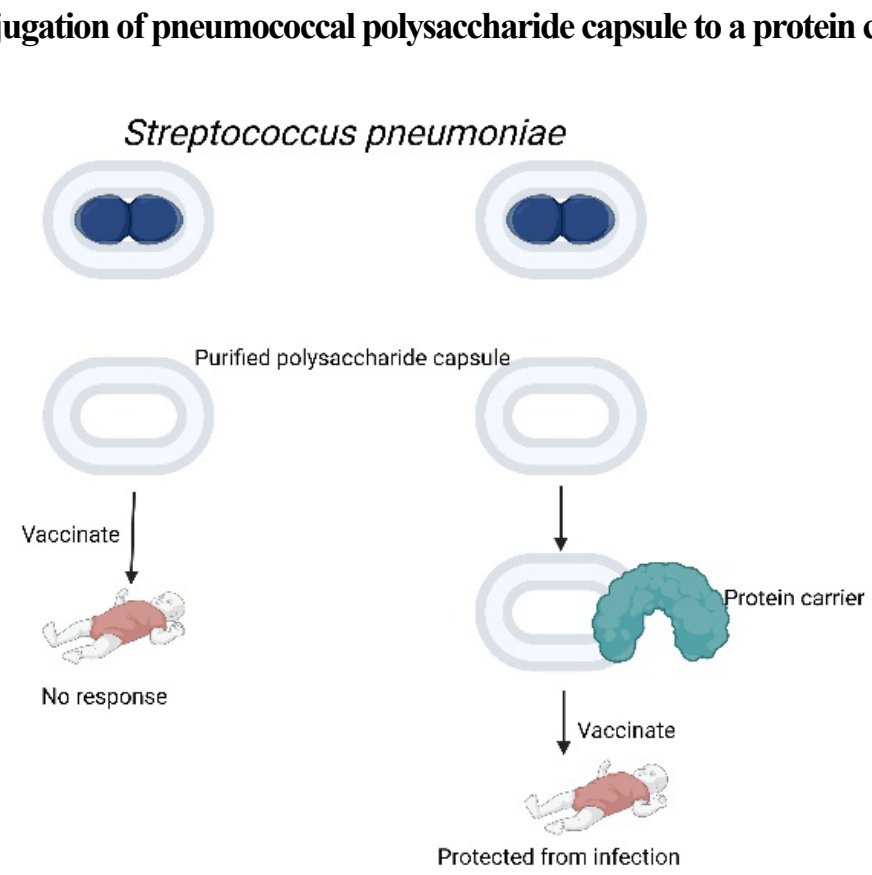

Figure 1. Conjugation of pneumococcal polysaccharide capsule to a protein carrier. Infants less than two years of age fail to respond to purified polysaccharide vaccines. Conjugation of the polysaccharide to a protein carrier elicits protection in young children.

There are at least two other challenges for subunit vaccines. In most cases subunit vaccines do not elicit as strong of a response as immunizing with an entire microbe. Thus, subunit vaccines maybe less efficacious as compared to immunization with entire microbes. To increase the efficacy of a subunit vaccine, an adjuvant can be added to the vaccine preparation. An adjuvant is a substance that can increase the potency of an antigen (subunit). Often adjuvants are derived from natural sources. When combined with an antigen, there can be a significant increase in the immune response to the antigen. Figure 2 shows a typical subunit vaccine formulation. There continue to be new developments in adjuvants. Another challenge with subunit vaccines is that immunity may wane over time which can result in the need for a booster. A booster can strengthen protection against the targeted pathogen. Such is the case with the tetanus vaccine. The first booster is recommended between ages 11 to 12. Thereafter, individuals should get boosters of the tetanus vaccine every ten years. Despite such challenges, subunit vaccines have been highly successful and are a mainstay of disease prevention.

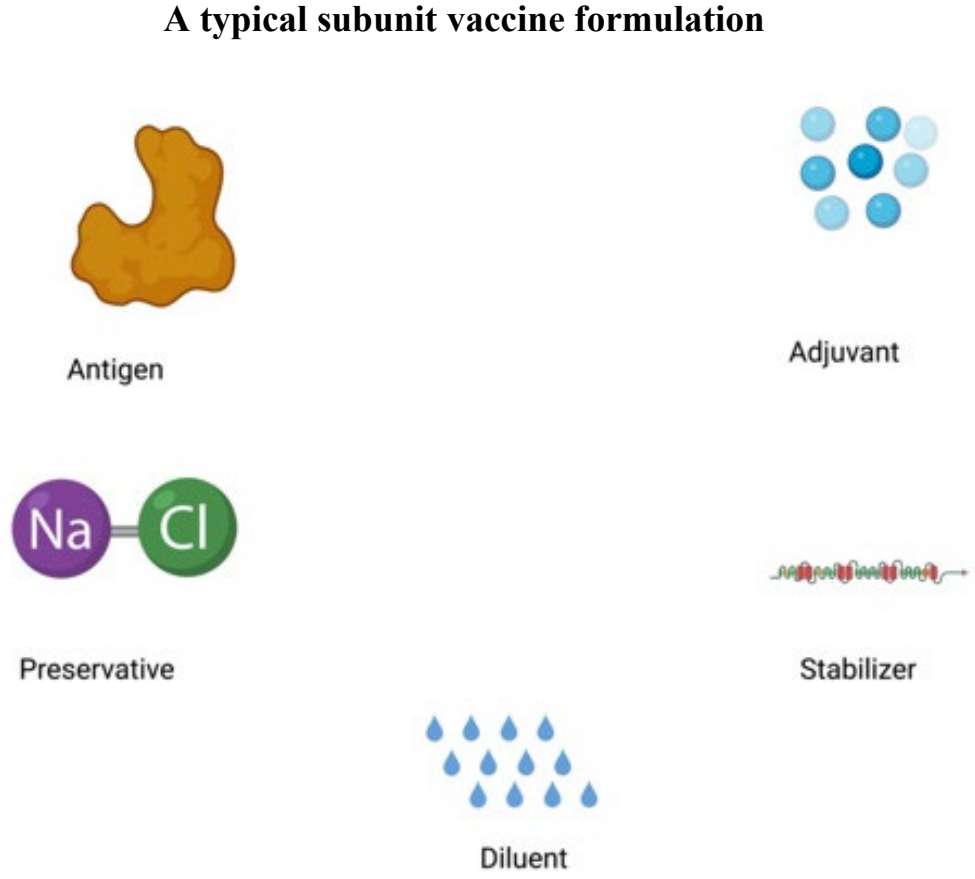

Figure 2. A typical subunit vaccine formulation. A purified antigen is combined with an adjuvant to elicit a robust immune response. A preservative, stabilizer, and diluent are added to the formulation.

The challenges of traditional vaccines such as those seen with subunit vaccines have resulted in the development of new vaccine technologies. Molecular biology approaches have fostered new vaccine approaches. This includes the use of viral vector vaccines (Tatsis and Ertl, 2004;) and nucleic acid vaccines (Fomsgaard and Liu, 2021;). Viral vector vaccines use a modified virus to deliver nucleic acid to cells. The vector contains DNA encoding an antigen for the infectious agent. In this approach neither the viral vector nor the transported DNA cause an infection in the person being immunized. Importantly, the delivered genomic material does not integrate into the genomic acid of the recipient. The genes necessary for the replication of the viral vector have been removed. The transported DNA expresses the antigen in the recipient's cells. This induces a strong cytotoxic T cell response that is effective in preventing viral infections. This contrasts with subunit vaccines, which elicit a more robust antibody response. The most frequently used viral vector is adenovirus. Adenovirus vectors can efficiently introduce transported DNA into a cell, and the viral vector has a broad range of cells into which the virus can introduce transported DNA. Many people already have existing antibodies against adenovirus that can prevent the vector from delivering the transported DNA. This has been overcome by using adenovirus serotypes that fewer people have existing antibodies against. Several adenovirus-based vaccines have been approved for human use. This includes COVID-19 vaccines and vaccines for Ebola virus.

Nucleic acid vaccines, also known as genetic vaccines, have been under development for nearly 30 years. These vaccines contain genetic information (DNA or RNA) that encodes an

antigen from the infectious agent that is being vaccinated against (Liu, 2019;). DNA vaccines were the first genetic vaccines to be developed. These were plasmids containing DNA encoding an antigen from the targeted pathogen. When injected cells would take up the plasmid and process the nucleic acid to express the antigen on the cell surface. The cell surface expressed antigens are from a bacterium or a virus and recognized as foreign. This foreign antigen on the cell surface triggers the immune system which results in an immune response that can protect against the pathogen (Bosarge et al., 2011;). These DNA vaccines are considered to have no risk of infection, be easy to produce, stable, and cost-effective. DNA vaccines are available for veterinary use. However, DNA vaccines for human use are not available in the United States. A COVID-19 vaccine produced in India is the first DNA vaccine approved for human use (Khobragade et al., 2022;). Interest and research efforts remain high for human DNA vaccines. Studies focus on increasing the immune response elicited by DNA vaccines in humans.

mRNA vaccines obtained considerable attention since they were successful against COVID-19. These genetic vaccines are efficacious, not infectious, the mRNA does not integrate into the host cell, and the mRNA is degraded. mRNA vaccines have been under development since 1990 when it was demonstrated that mRNA injection into animals resulted in gene expression from the naked mRNA (Wolff et al., 1990;). mRNA injected genes are expressed in the cytoplasm of a cell by ribosomes. This is more efficient compared to DNA vaccines because the DNA not only has to be taken up by the cell, but it also must cross the nuclear membrane for expression to occur. Subsequent studies demonstrated that incorporating mRNA in lipid nanoparticles (LPN) increased the stability of the mRNA and uptake by cells. This can lead to higher gene expression and an increase in the immune response (Figure 3).

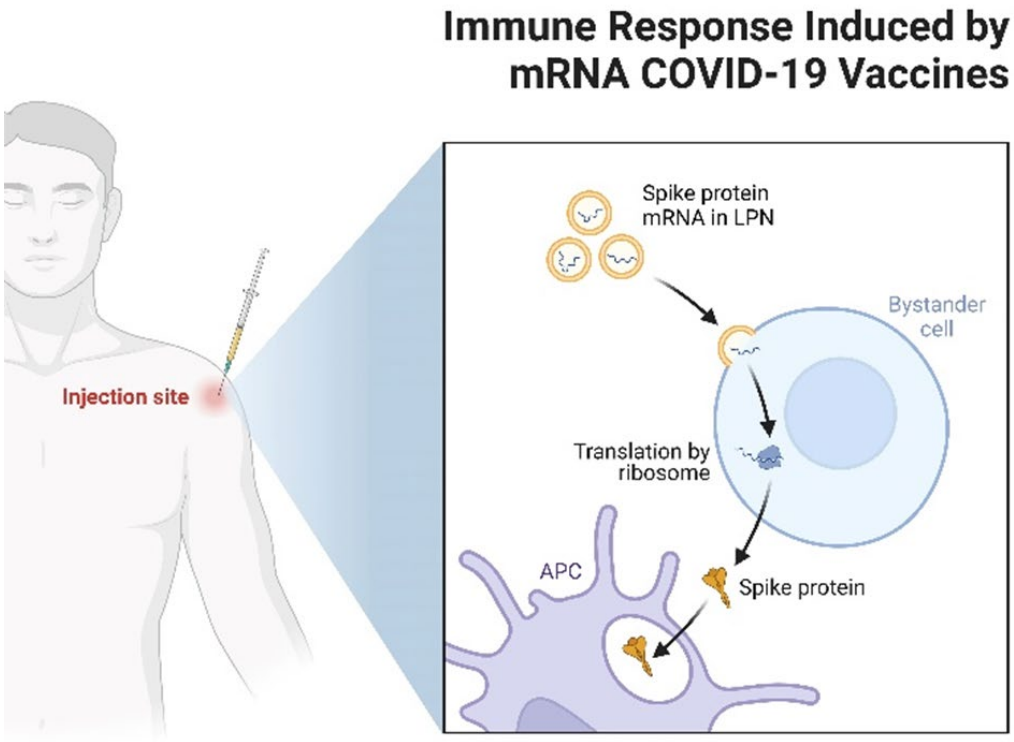

Figure 3.mRNA vaccine for COVID-19. The mRNA encoding the viral spike protein is enclosed in lipid nanoparticles (LPN). This protects the mRNA and increases delivery to cells. The spike protein is synthesized and taken up by antigen presenting cells (APC) which leads to a protective immune response.

It may have appeared that COVID-19 mRNA vaccines were rapidly developed without supporting studies. This is not the case as mRNA vaccines have been in development for more than 20 years. At the beginning of the COVID-19 pandemic, there were already several mRNA vaccines in various stages of clinical testing. Therefore, a lot of data on the safety and efficacy of mRNA vaccines was available. Given how easy it is to produce an mRNA vaccine, not much time was required to develop a spike protein vaccine and asses it's safety and efficacy in humans.

Novel future vaccine development

Vaccines were developed to prevent infectious diseases. However, vaccines are being explored for possible use in precision medicine for targets other than human pathogens. Probably the most promising non-infectious agent vaccines being developed are cancer vaccines. The problem with most cancers is that cancerous cells can very closely appear like normal cells of the body. This contrasts with pathogens which are seen as foreign by the body. Several targets have been identified on cancer cells that allow the host immune system to recognize and eliminate the cancer cells. Injection (vaccination) with these markers take place after the cancer has developed. Therefore, these are considered therapeutic cancer vaccines.

There are also preventive cancer vaccines. For example, it is known that human papilloma virus (HPV) can cause cancer in some individuals following infection with HPV. Thus, immunization with the HPV vaccine could prevent development of some cancers.

There are other novel vaccines under development. This includes vaccines targeting drug abuse. The vaccine would train the immune system to respond to and neutralize specific illegal drugs. There are studies on vaccines against atherosclerosis that could prevent strokes and heart disease. There is even the possibility of contraceptive vaccines. The vaccine could target sperm specific proteins. The vaccine would be reversible since data shows that the immunity would decline over time.

In addition to novel vaccines, studies are focused on improving delivery of vaccines. Some people have real fears of injections. Therefore, transdermal delivery of vaccines is being investigated. A patch containing the vaccine antigen would be applied to the skin so that the antigen would cross the skin without the need for injection. Other approaches incorporate the vaccine antigen into food. This would be of benefit in developing countries were transporting, storing, and administering vaccines can be challenging.

Engineering approaches are also playing an important role in improving vaccines. Combing antigen with material science techniques could improve the efficacy and safety of a vaccine. One approach is to combine antigen with molecular adjuvants or inflammatory singles. This could augment the immune response and increase the level of protection.

Vaccine dosing is also under investigation. The concept of precision medicine is to address the needs of the individual. Clearly, there is significant variation among individuals. Yet vaccines have often taken a "one size fits all" approach. A vaccine dose given to a 300-pound 60-year-old male will elicit a very different response compared to the response in a 20-year-old 110-pound female. Additionally, nutritional status, smoking, and underlying medical condition can influence immune responses to vaccines. Vaccine development continues to improve, but many factors can affect the desired outcome of protection against infection.

Hesitancy to anti-vaccination

Often anti-vaccine is seen as vaccine hesitancy. However, these two concepts are very different. A person who has not decided to get a vaccine for them self or their child is hesitant. In contrast, anti-vaccine advocates refuse to get vaccines and

typically try to influence others not to get vaccines.

Several factors can contribute to vaccine hesitancy. Complacency can contribute to an individual failing to get vaccinated. Complacency can occur when someone takes a "wait and see" attitude toward a vaccine. This approach has its risk. This approach can slow progress toward controlling an infectious disease. This has been the case with the COVID-19 vaccine. Waiting supersedes our ability to control the ongoing infections with SARS-CoV-2. Convenience can be another factor in hesitancy. Time off from work or travel to a vaccine site can result in someone hesitating in getting the vaccinated. Involvement and understanding by employers can go a long way to reduce anxiety and increase employees obtaining vaccines. This can lead to an improved workplace environment.

Vaccine misinformation can lead to hesitancy and vaccine refusal. A recent study showed a negative relationship between online misinformation and vaccine acceptance (Pierri et al., 2022;).Social media misinformation is widespread. This has caused a significant decline in public trust. The CDC has defined misinformation as false information that is not intended to mislead others. Disinformation is created deliberately to mislead others. Clearly both can erode public confidence.

In contrast to hesitancy, the anti-vaccine groups have developed strong backing over the last couple of decades. As long as there have been vaccines, there was anti-vaccine groups against the vaccine. The first widely used vaccine derived from cowpox material protected against smallpox when used to immunize children in the late 1800s. The anti-vaccine group claimed that injecting cow material into humans could cause them to develop characteristics of a cow. This disinformation resulted in many people not taking the smallpox vaccine. However, in the 1990s the anti-vaccine movement significantly increased. This was due to celebrities speaking out about unfounded complications resulting from vaccines. Subsequently, social media has provided a platform for anti-vaccine advocates.

Vaccines have become inseparable from politics. This makes the trust of science more difficult. Politically motivated denial of science threatens the ability of the public to make valid choices about vaccines. When politically motivated leaders provide disinformation, the public are deceived. Also, when erroneous information results in policy decisions this can result in public harm.

CONCLUSION

Precision medicine aims to prevent disease rather than intervene in an ongoing disease. Vaccines can effectively prevent infections or lessen disease severity during an infection. Rapidly changing technology has resulted in new levels of vaccine development. Vaccine science has saved countless lives and can prepare us for future pandemics. However, racial and ethnic disparities influence vaccine uptake, and vaccine preventable diseases affect poorer neighborhoods. There have always been controversies regarding vaccines. The current political divide has negatively affected vaccines and science. Challenges exist, but the overwhelming evidence supports the broad use of safe vaccines.

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Opening New Pathways to HPV Cancer Prevention through Changes in Practice, Policy, and Partnerships

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ABSTRACT

Introduction: In the U.S., an estimated 46,143 Human Papillomavirus (HPV)-associated cancers occur each year. (CDC, 2021;). HPV vaccination and cancer disparities exist geographically and by race and ethnicity. Cancer sites include the cervix, vulva, vagina, penis, anus, and oropharynx. HPV vaccination may prevent 90% of cervical cancers if vaccinated at the recommended ages of 9-12, even without screening. Of the HPV infections that persist and evolve to cervical cancer, 80% were acquired before age 26. In 2018, across the U.S., HPV vaccination completion rates for girls and boys were the lowest in Mississippi. Lack of vaccine uptake prevents community-acquired immunity from reducing disease and death. Therefore, the goal was to create statewide partnerships to improve HPV vaccination.
Methods: The American Cancer Society (ACS), the Mississippi State Department of Health (MSDH), the University of Mississippi Medical Center (UMMC), and many statewide partners formed alliances. In 2019, a Mississippi HPV Roundtable (MS-HPV-RT) was created, and the MS-HPV-RT consists of experts from multiple sectors. Priority areas include: (1) strengthening provider recommendations, (2) engage systems change, and (3) increase community knowledge.
Results: In 2016, a new awareness of HPV cancer prevention developed and lead to improvements. In 2019, 1,853 more HPV vaccinations occurred in Mississippi than in 2018, a 69.2 % increase after widespread system changes. Effective 2021, Mississippi Medicaid extended coverage of vaccines in pharmacy settings, and the Mississippi Board of Dental Examiners approved new dental privileges to vaccinate and bill. State Health Officer Resolutions and Legislative endorsements created observances.
Discussion: Adolescent vaccination is the key to HPV cancer prevention; however, screening remains critical to preventing HPV-related cancers. In the U.S., information is lacking on HPV vaccination programs and policies at the population level. Our study demonstrates changes in HPV vaccination practice and policies through statewide partnerships in Mississippi.
KEY WORDS – Cervical Cancer, Cervical Cancer Screening, Human Papillomaviruses (HPV), Vaccination, Oral Health, Dentists.

INTRODUCTION

In the U.S., an estimated 46,143 Human Papillomavirus (HPV)-associated cancers occur each year. (CDC, 2021;). In Mississippi, during 2015-2019, 2478 new cases of HPV- associated cancers were reported (CDC, 2021;). Of these cancers, over 90% could have been prevented with the 9-valent HPV vaccine (CDC, 2022;). However, nationally, adolescent vaccine uptake and completion rates remain too low. In 2018, in Mississippi, HPV up-to-date vaccinations rates were only 28.8%, *the lowest*

in the nation (Walker, 2019;)(See Figure 1). Reduced vaccine uptake fails to achieve the full potential of cancer prevention. Further, there is a lack of information on vaccination programs and policies at the individual and population level and on HPV prevalence (Hirth, 2019;).

National Immunization Coverage

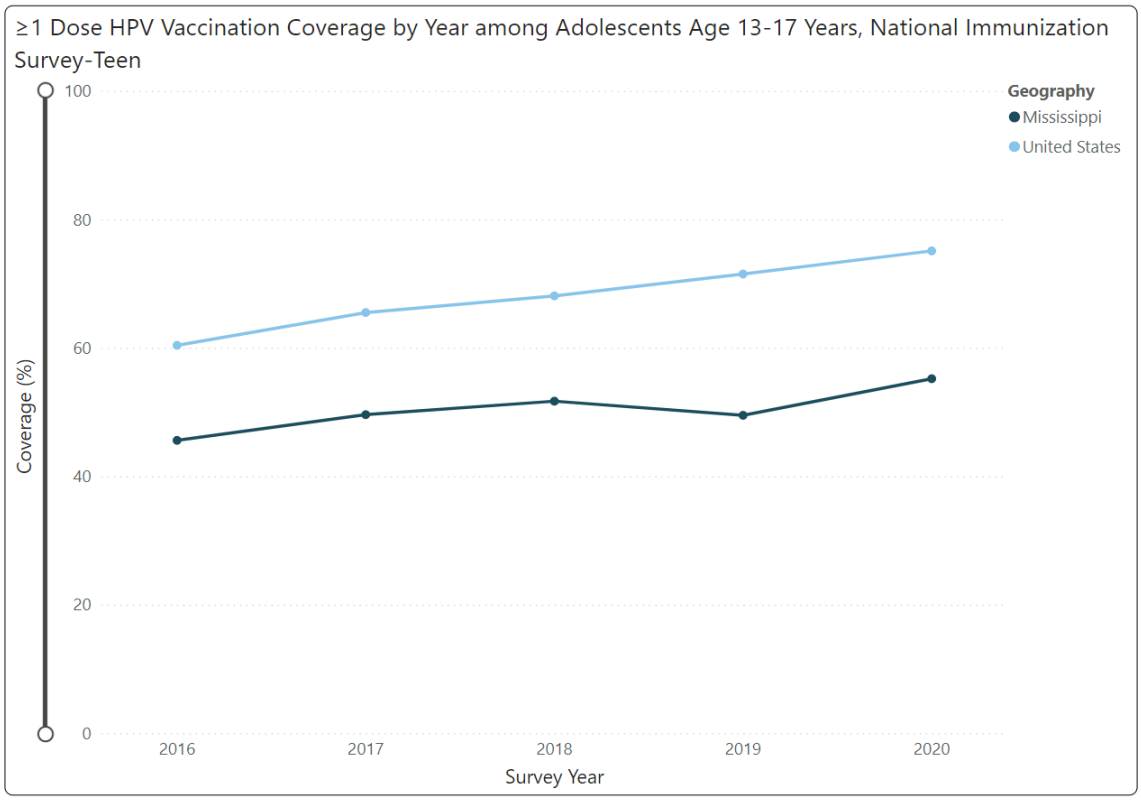

Figure 1. Illustration of National Vaccination Coverage, comparing Mississippi vs. United States. Adolescents aged 13-17 years received ≥ 1 dose of HPV vaccine within a year. Increases from 2018-2020 years, in ≥ 1 dose of HPV vaccine coverage were observed.

HPV vaccination prevents multiple types of cancers. Persistent infections by high risk HPV cause cancer of the cervix, vulva, vagina, penis, anus, and oropharynx (back of the throat, including the base of the tongue and tonsils) (ACS, 2022;). In a CDC study, HPV was attributable to 70% of all oropharyngeal cancers and 90% of cervical cancers (CDC, 2021;). Importantly, HPV-associated cancer affects males and females alike.

Approximately 82% of oropharyngeal cancers occur in males (CDC, 2018;). Of the HPV infections that persist and develop into frank cervical cancer, 80% were acquired before age 26 (Hirth, 2019). HPV vaccination may prevent 90% of cervical cancers if vaccinated at recommended ages of 9-12, even without screening (Hirth, 2019;).

In 2006, the FDA licensed the first HPV vaccine and subsequent versions followed in 2009 and 2014. Now, over 135 million doses have been distributed and monitored for safety (CDC, 2022;). The updated CDC Advisory Committee on Immunization Practices (ACIP) guidelines has been updated to clarify that if the vaccination schedule is interrupted, the series does not need to be restarted (Wodi et al.,

2011;). More than 15 years of reassuring research data point to safe and effective cancer prevention.

The public's hesitancy and acceptance of HPV vaccination have not matched that of other immunizations (AACR, 2021;). Rural areas have lower completion rates than urban (Hirth, 2019;). When comparing race and ethnicity, Black females aged 13-17 were more likely to be HPV vaccinated than White females (CDC, 2022;). Further, those at or below the poverty level were more likely to be vaccinated; success is credited to Vaccines for Children (VFC) (Hirth, 2019;). Conversely, the disparity in low vaccination is tied to high-income individuals, who are more likely to have private health insurance.

Together, leaders from the American Cancer Society (ACS), the Mississippi State Department of Health (MSDH) agency, and the University of Mississippi Medical Center (UMMC) formed statewide alliances in Mississippi to increase adolescent HPV vaccination rates. Multiple HPV task forces were created, then teams built a united platform. Led by the ACS, the Mississippi HPV Roundtable (MS-HPV-RT) coalesced to improve HPV cancer prevention and control. Herein, the goal is to describe the steps undertaken by statewide Mississippi partners to increase adolescent HPV vaccination and the strides together 'in the fight against cancer'.

METHODS

American Cancer Society (ACS)

In 2018, the ACS targeted education toward Pediatricians and Family Medicine clinicians using the ECHO model (The University of New Mexico, 2021;). The ECHO model brought experts and learners together to collaborate and problem-solve on improving provider recommendations for HPV vaccination. Best practices were deployed to clinicians to strongly endorse vaccination of boys and girls starting at age 9 and completion before age 13.

MS HPV Roundtable Forms

In 2019, the American Cancer Society created the Mississippi HPV Roundtable (MS-HPV-RT). The ACS Roundtable comprises experts from multiple sectors - public, private, industry, insurance, professional societies, and academia. Priority areas formed to (1) strengthen provider education and recommendations, (2) engage systems change, and (3) increase parent and community knowledge. A MS HPV RT website was created through funding from Southeast Louisiana Area Health Education Center (SE LA AHEC); https://www.mshpvroundtable.org/.

The MS HPV Roundtable Partner Organizations include: American Academy of Pediatrics- M.S. Chapter, American Cancer Society, American Association of Public Health APHA-ASP MS chapter, Baptist Hospital, BCBS of M.S., Central MS Health Services, Coastal Family Health, Community Health Center Association of M.S., Division of Medicaid, Dept of Education/School Nurses, GA Carmichael Family Health Center, Hattiesburg Clinic, Humana, Jackson-Hinds Comprehensive Health Center, Magnolia Health Plan, Mary Bird Perkins- Natchez, Merck-Vaccine Policy & Government Relations, Molina Healthcare, MS Academy of Family Physicians, MS Cancer Registry, MS Congregational Nurses, MS Hospital Association, MS Medical Association, MS Medical and Surgical Association, MS Nurses Association, MS Oncology Society, MS Pharmacy Association, MS Public Health Association, MS Rural Association, MS Department of Health, MSU Ext Service, Plan A Health, Quinn Total Health, Southeast Louisiana Area Health Education Center (SE LA AHEC), Senator Wickers office, Singing River Health System, Southeast MS Rural Health Initiative, Inc., St. Dominic Hospital, St. Jude Children's Research Hospital, Teen Health MS, Trinity Pediatric Clinic, UMMC, UMMC- ACT Center for Tobacco Treatment and Education, UMMC- School of Dentistry, UMMC – School of Medicine, UMMC- School of Nursing, United Healthcare, University of M.S.- ACS on Campus Volunteers, University of M.S.- School of Applied Sciences, University of M.S. - School of Pharmacy, Walgreens Pharmacy, Walmart Pharmacy. The following paragraphs highlight some recent methods that began after the ACS MS HPV Roundtable was formed.

Mississippi State Department of Health (MSDH)

In 2016, MSDH agency leaders identified gaps of knowledge concerning HPV-related cancers and HPV

vaccination in both the public and their staff. An MSDH HPV task force was formed. A comprehensive system-wide plan and methods to revise policies and procedures to increase immunization were instituted. New public emphasis and strategies on creating a "culture" of HPV Oral Health Awareness began for cancer prevention.

MSDH HPV ORAL HEALTH

The MSDH Office of Oral Health took a grassroots approach to population-based oral health promotion, oral disease prevention, and policy implementation. A top-down education strategy, outreach, and interventions at every level were deemed required for the public and dental workforce to understand the benefits of HPV vaccination for oropharyngeal cancer prevention.

HPV disease prevention and oral health promotion were amplified through integrated efforts of interdisciplinary teams, including the Bureaus of Health Services, Field Services, and Preventive Health. Next, the task force increased its work external to the agency. Regional oral health consultants (dental hygienists) participated in immunization clinics by creating educational stations and care coordination to a dental home. In addition, strategic trans-sector collaborations evolved between the MSDH, the UMMC School of Dentistry (SOD), and the Mississippi HPV Roundtable MS HPV RT.

Groundbreaking methods first created the tagline "Creating a Culture of Oral Health." This tagline was a product of State Health Department Priorities (Create a Culture of Health) and State Oral Health Plan (2016-2022).

Partnerships with the Mississippi Dental Association (MDA) and Mississippi Dental Society (MDS) increased by way of the MSDH Oral Health Updates letter, District meetings, Annual conferences, and article submissions to MDA for submission to dental providers throughout the state. Members partnered for events with local hospitals, community health centers, and dental hygiene schools to provide education, oral health cancer screening, and referral to specialty care.

Utilizing national frameworks, HPV cancer prevention and oral health messages were communicated across the life span to diverse audiences (Shukla et al., 2022;) Information was disseminated through office campaigns, webpages and social media, and banners at community events. In addition, official national and state health observance days highlighted the impact.

MSDH System Changes in Vaccination

In Mississippi, all incoming seventh graders must receive a Tdap booster as a required vaccine for school entry. (Approximately one-fifth of all incoming seventh graders receive the Tdap vaccine through MSDH county health departments.) Back to school Tdap vaccinations served as an excellent opportunity to tri-vaccinate adolescents against HPV and meningococcal meningitis.

Beginning in 2018 to 2019, unprecedented system wide policy changes were implemented at MSDH to improve the uptake of each vaccine. Widespread changes began within the Office of Immunization, Nursing, Field Services, and Preventive Health teams with a summer Back-to-School campaign. Leadership required educational training created by the CDC and ACS for all staff participating in vaccination activities, including clerical and medical assistants.

Nursing staff scheduled specific Back-to-School campaign dates for "adolescent only" clinics to allow for more staffing. Nurses, clerical staff and pharmacists not typically engaged in clinic activity were deployed to work in the vaccine clinics to assist in higher volumes. Those less familiar with vaccine schedules helped primarily with vaccine education and declination forms. Critical to this effort was an approach that assured that all members of the team, from front desk staff to nurses and clinicians, were properly trained and educated to support parents in their decision process.

From a systems approach, new policies and procedure made the "best-practice choice", the default choice. A "declination form" was created that required the clinic staff to review **all** recommended immunizations and if the parent declined any of the vaccines, the form documented that the parents or authorized adult fully understood the choice to decline a vaccine. (Table 1.) The declination form tells adults that vaccine benefits *include cancer prevention*. New, directed staff messaging required nurses to inform the adult of **all**

vaccinations recommended at the time of the visit. Provider messaging to parents emphasized concurrent administration of not only the required school vaccination, but all recommended ones such as meningococcal and HPV.

Table 1. Sample Template for Parental Signature to Decline HPV Immunization.

Vaccine Declination Form
Decision to Not Vaccinate My Child
(THIS FORM IS NOT AN EXEMPTION FROM VACCINATIONS)
Child's Name: _____ Date of Birth: _____
Parent's/Guardian's Name: _____
A licensed vaccine administrator, _____, has advised me that my child should receive the vaccines indicated below. I have been provided and given the opportunity to read each Vaccine Information Statement (VIS) and/or the Emergency Use Authorization (EUA) explaining the benefits and risks of each of the vaccines recommended for my child. I have been given the opportunity to discuss all of the information given to me. However, I have decided not to have my child vaccinated at this time for the following vaccine-preventable diseases:

Vaccine/Disease	VIS/EUA given	Vaccine Recommended by (Nurse's initials)	I decline this vaccine (Initials of parent/guardian)
Human papillomavirus (HPV) - Adolescent: 11-18 years			
Meningococcal (MCV) Adolescent: 10-18 years			
COVID-19 – Adolescent 12-18 years			
Other			

I understand the following:

- The purpose of and need for the recommended vaccine(s)
- **HPV vaccine could help to protect my child from 6 types of cancer**
- The risks and benefits of the recommended vaccine(s)
- If my child does not receive the vaccine(s), the consequences may include:
  - Contracting the illness the vaccine could prevent
  - Transmitting the disease to others
  - The need for my child to stay out of school during disease outbreaks
- The American Academy of Pediatrics, the American Academy of Family Physicians, and the Centers for Disease Control and Prevention strongly recommended preventing these diseases through vaccination.
- Completion of this form does not provide my child with an exemption from school immunization requirements.
- Refusal to vaccinate may endanger the health or life of my child and others that come in contact to my child.

In signing this form, I acknowledge I am refusing to have my child vaccinated against one or more diseases listed above; I have placed my initials in the column titled "I decline this vaccine" to indicate the vaccine(s) I am declining. I understand that at any time in the future, I can change my mind and vaccinate my child.

Parent/Guardian Signature: _____Date: _____
Licensed Provider Signature: _____Date: _____

Community and school outreach included development and distribution of information to superintendents, school nurses, and community partners. Reminders to parents of 6th graders regarding required school vaccination and recommended vaccine schedules (Wodi et al., 2021;). MSDH nursing leaders presented at the School Nurse Conference to educate them on the importance of ALL adolescent vaccines, regardless of state school requirements.

HPV Summit

Funds from a Merck grant to the Mississippi Chapter, American Academy of Pediatrics supported an educational HPV Summit. In partnership with ACS and the MS HPV RT, provider-education on HPV-related cancers and vaccination were the focus, and continuing education credits were issued.

University of Mississippi Medical Center (UMMC)

UMMC School of Nursing (SON)

In 2018, the research study called STRIDES – **ST**udying **R**isk to **I**mprove Dispariti**ES** was initiated. The UMMC SON partnered with the National Institutes of Health (NIH) National Cancer Institute (NCI), and the MSDH to investigate cervical cancer screening and disparities **(**Risley et al., 2021;), including analyzing HPV infection, genotype prevalence, persistence, and progression to disease in those vaccinated and unvaccinated.

University of Mississippi Medical Center, Department of Pediatrics

The UMMC Department of Pediatrics quality initiative projects are underway. Educational hand-outs for families were developed as well as educational posters placed in the patient rooms. Staff and faculty education included methods highlighted by AAP in the HPV Summit. Methods include a shift in best practices from HPV vaccination recommendations starting at age 11, change to starting at age 9 and standardized processes for ordering and scheduling vaccine #2 with the ordering and administration of Vaccine #1.

University of Mississippi

In 2021, a multidisciplinary team at the University of Mississippi Oxford campus launched an HPV vaccine educational campaign. This campaign focused on increasing the awareness of HPV and the number of students vaccinated against HPV on campus. Coupled with social media advertising, a series of tabling events occurred around campus throughout the school year. Working with the student health center on campus, the student health pharmacy was able to administer the vaccine with incentives provided to students for every dose received.

Through grant funding from the MSDH Comprehensive Cancer Division, the HPV Free MS *Collegiate toolkit* was designed to be utilized by any college/university across Mississippi. Some of the components of the toolkit include a campus needs assessment, customizable graphics/social media posts for advertising, volunteer training materials, a guide for conducting effective tabling events, and standardized evaluation tools. For 2022, the campaign is expanding curriculum to include HPV vaccine education. The curriculum is being added to the freshman experience class, which is taken by every incoming freshman at the University of Mississippi.

ACS Health Plan Learning Collaborative

To increase U.S. adolescent HPV vaccination rates nationally to 80% by 2026, the ACS has launched a nationwide public health campaign called, Mission: HPV Cancer Free. The ACS established a Health Plan Learning Collaborative called HPV VACs (Vaccinate Adolescents against Cancer).

HPV VACs partners with health insurance providers to develop and evaluate quality initiatives within their organization. Analytic methods focus on improving quality metrics including the Healthcare Effectiveness Data and Information Set (HEDIS) adolescent immunization measure and eliminating missed vaccinations. Strategies focus on 1) Effective communication with different populations, 2) Providing HPV Roundtable materials, 3) Addressing Provider hesitancy and solutions, 4) Evaluating vaccine impact and rebound due to COVID-19, and 5) Vaccine initiation at age nine.

Insurance Providers and Partners

In Mississippi, health insurance providers Magnolia Health and United Healthcare (UHC) are participating systems in HPV VACs. Vital data analysis and outreach to insured community members and healthcare providers is conducted in each organization to increase vaccination. Formal analyses will be completed by early 2023. Each insurance provider augmented their provider approach to encourage them to begin administering the HPV vaccine as early as age 9 as a result of strategies learned at the HPV Summit.

Since 2021, UHC has implemented several new quality improvement projects aimed at increasing HPV vaccination rates for members. The quality improvement initiatives include: 1) Live and automated calls encouraging completion of wellness exams and immunizations, 2) Reminder calls to members needing a second dose of HPV vaccine, 3) Incentives for completion of adolescent vaccines, wellness visits, and specifically the HPV vaccine, 4) Reminder mail outs targeting members who need wellness exams and vaccinations, 5) Provider outreach and education.

Mississippi Division of Medicaid, Office of the Governor

Mississippi Medicaid enacted policy changes in late 2020 to allow licensed pharmacists to administer all vaccines listed on the Centers for Disease Control and Prevention (CDC) Immunization Schedules to beneficiaries. Pharmacy providers must be enrolled in the Vaccines for Children (VFC) program to administer vaccines to children.

RESULTS

MSDH HPV ORAL HEALTH

In April 2021, Mississippi Board of Dental Examiners (MSBDE) approved all licensed dentists in the state to provide vaccinations for HPV, COVID-19, Flu and Shingles under the direction that all license holders must abide by the MSDH and federal regulations. In March 2022, the American Dental Association (ADA) Coding maintenance committee approved the addition of HPV vaccination administration codes to the 2023 CDT Procedure Coding for Dentists. Dentists are now allowed to bill for vaccinations.

MSDH System Changes in Vaccination

In 2019, after widespread system changes, the MSDH reported a 69.2% increase in HPV vaccination for the 2019 back-to-school effort when compared with 2018. An additional 1,853 adolescents achieved HPV protection through this effort when compared with 2018.

HPV Summit

Best practices and educational and system strategies were delivered to a broad interdisciplinary audience of over 219 participants.

University of Mississippi Medical Center (UMMC) UMMC School of Nursing (SON)

In the Mississippi STRIDES cohort, a high burden of HPV positivity is present with significant racial differences in HPV genotype prevalence. **(**Risley et al., 2021;). Further, Black individuals in the cohort experience an earlier peak of high-grade Pap abnormalities compared to Whites **(**Clarke et al., 202;). The statewide population cohort research is ongoing.

UMMC Department of Pediatrics

The UMMC pediatricians report a favorable shift in the "culture" towards initiation of vaccination starting at age 9. Data collection and analysis is ongoing. Early notable increases with co-administration of Tdap and HPV and tri-administered Tdap, HPV and Meningococcal vaccines are also reported at UMMC.

University of Mississippi

University of Mississippi Oxford campus developed a toolkit, available free to any college/university. (More information about the HPV Free MS Collegiate Toolkit can be obtained by emailing (hpvroundtable@olemiss.edu.) Following the launch of the HPV vaccine educational campaign at the University of Mississippi in fall 2021, student health services saw a substantial increase in the number of HPV vaccinations administered during the 2021/2022 academic year compared to recent years. In 2019/2020, 12 doses were given, in 2020/2021 only 8 doses were given, however, in 2021/2022, 32 doses

were given through the pharmacy. For the 2022/2023 school year, the pharmacy has planned for an expected 50% increase.

ACS Health Plan Learning Collaborative

In 2019, HPV VACs partners report an overall 7.2 percentage point increase in HPV vaccine completion and an average increase of 11.3 percentage points in HPV vaccine initiation.

Insurance Providers and Partners

United Healthcare reports preliminary success with member and provider incentive programs and outreach. Magnolia Health reported member HPV vaccine completion rates in 2016 were 5.25% and improved to 21.7% in 2021. Across the nation, vaccination rates dipped in 2020 secondary to COVID-19.

Mississippi Division of Medicaid, Office of the Governor

Statewide policy changes became effective in March 2021; Mississippi Medicaid extended coverage of vaccines in the pharmacy setting. Vaccines administered by pharmacists do not count toward monthly Medicaid prescription limits and no copayments are required. Prior to this change, a very limited list of vaccines were open for coverage and billing by pharmacies.

Official Public Observances and Endorsements

In 2021 and 2022, new M.S. legislative bills and State Health Officer endorsements provided official health awareness days to amplify campaigns for HPV cancer prevention, http://billstatus.ls.state.ms.us/2021/pdf/history/SC/SC0532.xml. The MS State Health Officer resolution on March 4, 2021, was a call to attention to the connection between the HPV and cancer.

DISCUSSION

The HPV vaccine is cancer prevention. For example, New Zealand reported a 92% reduction in HPV infection and 70% in cervical precancer, crediting successful vaccination campaigns (Cancer Council Victoria, 2021;). Yet, in the U.S. overall, the Healthy People 2020 HPV vaccine target of 80% completion, was not met (CDC, 2022;). Provider recommendation is central to vaccine uptake and recommendations were reported to be the lowest, 59.5% in Mississippi when compared to 90.7% in Massachusetts (Walker et al., 2019;).

Vaccines are safe and effective to accelerate the decline of new cancer cases. Cancer incidence in men is declining nationally, but not in females. From 1999-2016, the annual average percent change (AAPC) for overall new cancer cases for females of all ages has not declined; the cancer incidence AAPC = 0.0 (95% CI = –0.1 to 0.1) (Ward et al., 2019;). In the southern U.S., including M.S., racial disparities in cervical cancer mortality are prevalent. Black females are disproportionally more likely to die from cervical cancer than White females; and the gap is greater in those over age 65 (ACS, 2022;). Hence, creating statewide and trans-sector partnerships to tackle low HPV vaccination is critical.

In Mississippi, grassroots efforts made inroads. However, the catalyst to population level cancer control and prevention comes through partnerships. The American Cancer Society united voices and accelerated the movement with the formation of the MS HPV RT. Better together, the community, local businesses, industry, insurance and healthcare providers have united with academia, state public health organizations, and national platforms. Although, the COVID-19 pandemic initially dampened progress, and most adolescent immunizations rates dipped nationally, the silver linings for the future of immunization are just being realized.

With the devastations presented by the COVID-19 pandemic, development of the COVID-19 vaccination and need to increase its uptake nationwide resulted in enhanced discussions to accelerate progress. Federal and state governments expanded vaccine privileges to other healthcare professionals and locations. Expanding the scope of care to new administration sites, including dental offices and pharmacies proved valuable.

Concurrently, reported HPV-related oropharyngeal cancer incidence rates began to exceed that of cervical cancer. In response, collaborative efforts were championed by the state health officer, the office of oral health, and the office of immunization teams.

Their team partnered with the M.S. American Dental Association (ADA) and the Mississippi State Board of Dental Examiners (MSBDE) to create a new "culture of oral health awareness" and expand practice and prevention.

Expert drivers of vaccination, AAP members and UMMC pediatricians are energized. The transition to earlier age of HPV administration at age 9 is regarded as a "culture and practice shift" successfully underway. College students also present a unique opportunity for HPV vaccination catch-up. A high prevalence of new HPV infections occur among teens and early adults. Therefore, the transition to college represents the first time many young adults are in charge of making their own healthcare decisions. Pharmacists at the U.M. report vaccine success stories on campus with their newly created college tool kit.

Incentives

Incentives coupled with convenient administration, and no cost on college campuses have shown increased uptake in vaccination. Insurance providers also credit incentives to their successes.

Limitations

Most private insurance plans now cover HPV vaccination for boys and girls; however, a wellness visit with a clinician and not a pharmacy visit may be required. One ***significant*** challenge for pharmacies is becoming a federal VFC provider to provide (free) vaccines for children. The VFC program provides vaccines at no cost to children who have the inability to pay. However, the program is built around clinics and not pharmacies. This makes it very difficult for pharmacists to comply with VFC. As a result, currently there are no VFC pharmacies in MS. However, pharmacies can still provide the vaccine, regardless of VFC, and it may be covered under another insurance option.

The ability for dentists to vaccinate began system statewide system changes. However, barriers arise for those offering vaccinations at their sites. Barriers are related to process issues and billing for services rendered. Becoming a vaccine provider is complex with regulatory compliance, storage requirements, and like processes that assure standard quality measure remain.

As such, the office of oral health has partnered with pharmacists in the state to discuss other service models of delivery that may assist others in providing vaccinations. Some of these models include dentist writing prescriptions and working with the local pharmacies to store said vaccinations.

Fortunately, federally qualified health centers (FQHCs) are strategic partners that can help fill the gap (Fisher-Borne, et al., 2018;). FQHCs often have medical and dental departments co-located. Some FQHCs share integrated electronic health records that provide fast access to the Mississippi Immunization Information Exchange MIIX and make work across disciplines easier to facilitate. Efforts are underway in Mississippi to pilot the administration of vaccinations by dentists in a primary care setting.

Lessons learned

Oral health posters on oral cancer were provided to health departments throughout the state. This message was not well embraced. Lessons were learned to focus discussions on cancer and not oral sex.

Misconceptions regarding HPV cancer, vaccination, and vaccine hesitancy abound. However, evidence supports that HPV vaccination is not linked to increase sexual activity. Tips for guided provider messaging can be located on the National HPV Roundtable website at https://hpvroundtable.org/ .

Screening for Cervical cancer

Evidence shows widespread HPV vaccine may prevent 90% of cervical cancers (NCI, 2022;). However, HPV vaccination rates fail to meet 80% coverage; the rate expected to provide community immunity. Cervical cancer screening must continue regardless of vaccination status because the vaccine protects against most but not all vaccine types (CDC, 2022;).

The United States Preventative Task Force (USPTF), the ACS, and the American Society of Colposcopy and Cervical Pathology (ASCCP) endorse cervical cancer screening (CCS) with primary HPV testing, co-testing, or cervical Pap (Smear) Cytology tests. Screening age recommendations include ages 21-65, depending on the screening methods. However, a common misconception perpetuates regarding the age

to stop CCS. Individuals aged 65 and older ***should*** continue screening until they have had at least 1 normal test in the previous 5 years and several tests in row from the previous 10 years (White et al., 2017; and CDC/cancer 2022;). If a person has had an abnormal result or treatment for cervical precancer or cancer, they should continue screening until advised by their clinician to stop.

Future steps to Prevent HPV Cancers

The UMMC Department of OB/GYN plans to offer postpartum HPV vaccination prior to discharge. The stalwart practice of Rubella vaccination after childbirth will be leveraged.

Additionally, the UMMC School of Nursing, the Department of OB/GYN, and the UMMC Department of Information Systems (DIS) are co-leading a "big-idea" initiative with the Center for Disease Control and the non-profit MITRE Corp (Saraiya et al., 2022;). The project's goal will integrate an electronic cervical cancer screening clinical decision support (CDS) tool into the electronic medical record (EMR). Current cervical cancer screening and management guidelines, including HPV vaccine reminders will appear on a clinician dashboard. This next generation digital tool will be open-sourced and put decision support at the fingertips of clinicians for informed shared-care cancer prevention.

CONCLUSION

The inclusion of HPV cancer control and prevention to overall health emerged through partnership opportunities in research, practice, and policy, education, training and working across disciplines. Pathways to system change prevail in the delivery of vaccinations statewide and translate to improved care access and strides towards reduction in cancer disparities.

In the U.S., one out of every 2 men and 1 out of every 3 women will be diagnosed with cancer in their lifetime (ACS, 2022;). Make an appointment now to vaccinate for 6 types of HPV cancer (ACS, 2022; NCI, 2022;). When booking a health visit, consider booking an HPV immunization visit for a child or grandchild.

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Role of Biological Markers in Kidney Transplantation

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ABSTRACT

Biological markers are considered a useful tool for predicting short-term outcomes and the incidence of allograft rejection episodes. Although recipient survival and kidney allograft have increased during the last decade, allograft survival rate beyond one year has not significantly improved. Risk factors linked with 1-year graft loss are associated with recipient/donor age, living vs. deceased donor, human leukocyte antigen (HLA) mismatch, cold ischemia time and delayed graft function (DGF). The effect of each factor although is small suggesting the need for predictive system that incorporates multiple features.

Current diagnostics for assessing allograft function/rejection through biopsy is invasive and may carry risk of significant complications. Advances in OMICs technology delivered the discovery of many candidate biomarkers associated with early stages of post-transplantation such as DGF as well as acute rejection episodes. Our study has two folds: first to review the literature within the last 5-6 years (2016-2022), mainly with the goal of non-invasive biomarkers associated with kidney transplant outcome. We selected those with non-invasive procedures that utilized either peripheral blood or urine specimens identifying molecular markers for prediction of allograft outcomes. Second to use databases of our own studies of genomic analysis of biomarkers mainly represented by gene expression signatures correlated with innate immune system. Gene series H19k and H8k used with mRNA from patient's whom experienced stable graft function (SGF) and those with multiple rejection episodes (RE). Biological markers were studied to identify early subclinical conditions associated with cold ischemia time and DGF.

Key words: Allograft Rejection, Biological Markers, Cytokines, Delayed graft Function, Genes, Inflammation, Toll-like Receptors, Transplantation

INTRODUCTION

Kidney transplantation is a treatment of choice for individuals with end stage renal disease (ESRD). The initial limitation in kidney transplantation were the development of techniques for vascular anastomoses, suitable donor organ, and managing the host immune response not to reject the transplanted organ. Mathieu Jaboulay and Alexis Carrel, visionary vascular surgeons and pioneers in organ transplantation, in 1906 attempted transplanting a goat kidney and later a pig kidney into patient with ESRD (Morris, 2004;). In both cases, transplants were unsuccessful.

Sometimes rejection occurs because of donor-recipient tissue-type incompatibility, other times because of cellular damage to the kidney, due to immunological and/or nonimmunological factors, after the brain death, as well as during organ recovery and procurement when the kidney is not receiving blood. This event causes an ischemic organ and ischemia reperfusion injury (IRI) which carries inflammatory markers, affecting kidney dysfunction and delayed graft function (DGF) (Perico, et al., 2004; and Mallon et al., 2013;).

Clinical outcomes particularly in early rejection and short-term allograft survival after kidney

transplantation have greatly improved due to advances in modern immunosuppression, tissue preservation, immunologic assessment and surgical techniques (Ponticell and Scolari, 2010; and O'Callaghan et al., 2012; Althaf et al., 2017;). In the United States (US), over the last 40 years, more than 30,000 organs were transplanted (Park et al., 2022;). The post-transplantation outcomes depend on multitude of disparities, thus remains difficult to predict. Acute rejection may appear in the first weeks after transplantation, when sudden pathological changes occur in the allograft, which are either T-cell mediated (TCMR) or antibody-mediated rejection (ABMR) (Hass et al., 2014;), but often is treatable using steroids and or anti-thymocyte-globulin (Wu et al., 2009;). However, acute rejection is a major risk factor associated with allograft failure and long-term graft survival (Kasiske 2011; and Schold 2022;). It has been observed that patients who recovered from early acute rejection may develop kidney interstitial tissue fibrosis causing chronic kidney disease. This is most likely associated with inflammatory cell trafficking and activation of innate to adaptive immunity (Chawla 2011; Sato and Tanagita, 2018; Albino et al., 2021;)

Multiple factors have been associated with long- term graft survival. This include donor/recipient type and characteristics, for example, HLA mismatch and pre-sensitization (high panel-reactive antibody), age, sex, race and comorbidities. Thus, long- term transplant outcomes are still a challenge particularly due to bacterial and viral infections, cellular rejection, course of immunosuppressive therapy etc., leading to chronic allograft rejection and allograft failure (Fishman and Rubin 1998; Bouamar et al., 2013; Hass et al., 2014; Hariharan et al., 2021;). Allograft survival rate beyond one year has not significantly improved, particularly in African Americans, late kidney allograft dysfunction remains a significant problem and more patients returning to dialysis as a choice for treatment. Risk factors linked with 1-year graft loss are associated with recipient's age, donor age, living vs. deceased donor, human leukocyte antigen (HLA) mismatch and delay graft function (DGF). Additional factors such as cold ischemia time, recipient pre-existing health such as diabetes, hypertension, pre-transplant BMI, cardiovascular disease have influenced on the outcome of allograft survival. The effect of each factor although is small suggesting the need for a predictive system that incorporates multiple features. Inflammation plays a fundamental role in terms of generating an immune response against the allograft. The immune response to allograft is complex and involves both innate and adaptive immune systems. A potential mechanism that triggers the inflammatory processes may occur early during an organ recovery and ischemia-reperfusion injury (IRI) (Land, 2007;). Early studies have suggested that IRI might initiate the release of inflammatory mediators associated with innate response boundaries (Albino et al., 2021;) resulting in inflammation and tissue fibrosis. The innate immune system is armed with dendritic cells, macrophages and complement cascade. Dendritic cells carry Toll-like receptors (TLRs), in which may recognize damage associated molecular patterns (DAMP) and cell debris released from kidney during IRI and become activated and mature. Figure 1, demonstrates innate and adaptive immune development due to damage to the transplant. It has been documented that ischemia (when arterial blood flow is interrupted) creates a structural and metabolic/cellular destruction causing organ contraction and tissue damage. Additionally, restoration of blood flow during the reperfusion of the donor organ, although needed it further causes tissue injury. We and others have reported association of cytokines and TLRs in association with allograft rejection (Hribova et al., 2005; Nogueira et al., 2010; McDaniel et al., 2013;). Thus, potential therapeutic strategies to inhibit activation of the innate immune event is needed to overcome kidney injury and further T- cell and B- cell mediated rejection. Furthermore, numerous biological markers derived from "OMICs" innovation which are considered a basis of precision medicine, have been the focus of investigation to improve the power of monitoring allograft outcome (Califf, 2018; Stapleton et al., 2018;).

Studying kidney transplantation progression, biological markers are useful tool for predicting clinical outcomes throughout the various phases of transplantation. Conventional biomarkers including HLA (human leukocyte antigen) genotypes although less powerful are reliable for identification of the

donor-recipients level of compatibility which are influential for predicting early rejection episodes and the incidence of delayed graft function. Kidney biopsy remains the gold standard for diagnosis of the allograft status. However, the procedure is invasive and carries variability because of sampling error and pathological validation (Huang and Farkash, 2016;). Current advances in genomics, have facilitated novel candidate biomarkers, particularly associated with the complexity of the immune parameters and can be used to detect sufficiently advanced subclinical injuries influencing rejection episodes. Nonetheless clinical implementation of novel biomarkers is challenging and only few have met clinical utilization. In recent reviews, Naesens et al., 2018; and Quaglia et al., 2020; elegantly described the general features and definitions of biomarkers in kidney transplant. In addition, Singh et al., 2018; reviewed targeted biomarkers in the context of T and B cells mediated rejection, using the state of art commercially available molecular platforms including Gene Chips HiSeq and various biological fluids including blood and urine for prediction of kidney allograft outcomes. Furthermore, various reviews have introduced single cell RNA sequencing (scRNA-seq) for mapping of different types of cells in the human kidney with the goal of better understanding cellular interaction and networking with disease status and the development of therapeutic targets (Menon et al., 2017;).

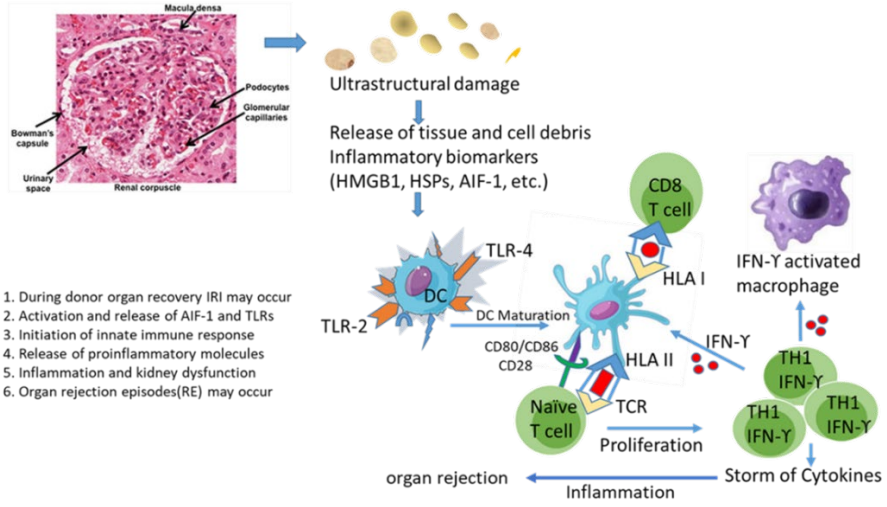


Figure 1. A graphic presentation of innate and adaptive immunity development due to damage to the transplant. Ischemia reperfusion injury (IRI) causes damage of tubular cells and release of damage associate molecules (DAMP) and cell debris. These molecules bind to TLRs on dendric cells (DC) and initiate the immune activation. Abbreviations: AIF-1, allograft inflammatory factor-1; TLRs: toll-like receptors; HMGB1: high mobility group box1; HSPs: heat shock proteins; HLA: human leukocyte antigen, TH1 T-helper cells; TCR: T-cells receptor; etc.

Given the molecular profiles of rejection episodes after kidney transplantation, notably the presence of genes associated with immunological parameters provides evidence that the involvement of the innate immune response in the initiation and extension of inflammation, may dictate the fate of transplantation outcomes. Our goal was to explore the significance of genomic markers in early rejection vs. late rejection episodes in association with cold/warm preservation time that may cause DGF. Prediction and prevention of DGF may play a role in long-term allograft survival.

METHODS

We have reviewed the available papers published within the last five-six years and our own database on the role of biomarkers in kidney transplantation. We selected studies that utilized various sample types, either peripheral blood or urine and molecular diagnostic methods for prediction of allograft outcomes (see Table 1.) A literature search using PubMed (NCBI/NIH) and http://www.ncbi.nml.gov/geo for identification of differential expression of signaling pathways in association with

immune response and disease status were performed. In addition, pathway analysis on microarray data sets using a well-established gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) approaches. We used gene enrichment analyses to determine interconnectivity between the genes/proteins (Menon et al., 2017; Marx D et al., 2019;) for their association with innate immunity pathway.

Patients

Informed consent was obtained as part of the protocol approved by the University of Mississippi Medical Center (UMMC) Review Board for drawing blood samples and studying biopsy specimens from each patient. Blood samples were number-coded and deidentified by name. Clinical outcome data received from transplant data coordinator in the Department of Surgery. A total of 400 patients who received kidney transplants between 2006 and 2012 at UMMC were evaluated of which 390 were included in this study. Clinical data were stratified, including: DGF, patients required dialysis within the 7-10 days post transplantation; SGF (stable graft function); RE (rejection episode), on the basis of antibody mediated (ABMR) or T-cell mediated (TCMR) rejections. Three samples from each patient at different time intervals (pre-transplantation, day 3 and day 6 after transplantation) were tested. Rejection episodes were analyzed at 4 time points; <6 months, 6-12 months, 13-36 months and >36 months at post-transplantation.

RNA Isolation and Reverse Transcriptase Polymerase Chain Reaction

The RNA was isolated from peripheral blood mononuclear cells (PBMC) described previously (McDaniel et al., 2013;).

Immunohistochemistry (IHC)

Paraffin blocks of surgical specimens with known clinical diagnosis corresponding with the study objectives were used. The IHC procedure was previously described (Barker et al., 2010;).

Microarray hybridization

Gene expression profiling was performed using Array 900 MPX (Genisphere, Hartfield, PA). Gene series H19K and H8K derived from human ESTs (Expressed Sequence Tags) imprinted on a glass slide. The material derived from PCR amplification of Synthetic EST clones were in the form of double stranded DNA and coupled to the slide matrix (*http://www.microarrays.ca*). The slides used in a sandwich technique (H19K vs. H8K) for hybridization of each set of samples. The cDNA microarray developed from PBMCs of patients with rejection episodes (RE) or stable graft function (SGF). Ten PBMCs samples from patients with either RE or SGF used for mRNA preparation in initial unsupervised, hierarchical clustering, described in another unpublished manuscript. Then the enrichment of specific functional groups of gene in our data was assessed using hyper-genomic distribution method (Jakt et al., 2001;). Using this criterion, a list of 208 genes identified. Gene-ontology-response to stimuli showed a majority (24.05%) of genes were associated with immunological parameters. The distribution is given in Table 2. We extracted only the gene subset of interest with expression levels of innate immune response genes such as Toll-like receptors (TLR2-TLR4) MyD88, and Cytokines in association with DGF, RE, CTI and returning to dialysis (RD).

Statistical Analysis

Patient's age, race, gender and presenting clinical data were analyzed using a SAS program to determine the effect on the clinical outcomes. Average eGFR was calculated at one-year post-transplant, and based on the observed data, patients were stratified into 2 groups, those who had the mean eGFR ≥35 mL/min/1.73 m^2, and those who had ≤35 mL/min/1.73 m^2. The group differences for mRNA transcript levels between GFR values were determined by one-way analysis of variance. Specificity levels of allograft inflammatory factor 1 (AIF-1), interleukin-18, toll-like receptor-2 (TLR-2), toll-like receptor-4 (TLR-4) were calculated on the basis of false positive vs. pathologic grades. *P*-values were computed from the Fisher exact test for categorical variables (counts and percentage) and Mann-Whitney *U* test for nonparametric continuous variables (means and SDs). The level of significance was set at $p<0.05$ for comparison between the groups.

Table 1. A selective summary review of biomarkers in kidney allograft transplantation outcomes from 2014-2022

Author	Sample type	Sample #	Analysis	Platform	Dysfunction	Study results	Literature cited
Kurian SM et al.	Blood	148pts	mRNA	Microarray	AR vs. ADNR	Distinguish AR & ADNR	Am J Transplant 2014; 14:1164-1172.
Gunther OP et al.	Blood	40pts	mRNA/ peptide	AffiyU133/ Mass spect	AR	Multi-omics data sets were predictive for monitoring AR	Omics A J. of Integrative Biology 2014;18:682-895
Thareja G et al.	Biopsy	40pts	mRNA	HiSeq 2500	TCMR/ active and chronic ABMR	Het/Hom ratio may offer quantitative parameters for TCMR & ABMR	Am J Transplant 2018;18:2429-2442
Nissaisorakarn V et al.	Urine	485 pt/ 4300 sp.	mRNA	RT-QPCR	ACR	Urinary cell mRNA profiles are informative of kidney allograft status	Human Immunol2018 79:343-355
Janssen M et al.	Blood	175pts	DNA	SNP Lightcycler 480	AR	HLA mismatch is significant risk factor and polymorphisms seem to be a crucial factor for prevention of AR.	Human Immunol2019 80:176-183
Hruba P et al	Biopsy	18pts	mRNA	Microarray	ABMR	CD46 and CD59 complement regulatory transcript/proteins increased in ABMR.	Transplantation 2019; 103:909-917
Morath C et al	Serum	1,724	PRA	HLA panel testing	DGF	Pre-transplant HLA Abs are predictive factor for DGF and impaired graft outcome.	Frontiers in Immunology 2020; doi:10.3389/
Malone AF et al.	Single cells from biopsy	81,139 cells	mRNA	HiSeq	ABMR and RE	Single cell- RNA sequencing of allograft identifies transcriptional profiles of donor recipient-derived cells in ABMR and non-rejecting states.	JASN 2020; 31:1977-1986
Thieme CJ et al	Urine Blood	26 pts	TEC PBMC	TreaT-Assay	AR	Pre-RTx donor-reactive T cells can predict the post-RTx AR	Nature Research 2019; 9:19037 www.nature.com/scientificreport

AR: acute rejection; ADNR: acute dysfunction no-rejection; TCMR: T-cell mediated rejection; ABMR: antibody mediated rejection; HLA: Human leukocyte antigen; DGF: delayed graft function; TEC: tubular epithelial cells; PBMC: peripheral blood mononuclear cells; Trea-T Assay: transplant reactive T-cells.

Table 2. Gene Ontology classification for specific functional groups analyzing H19K-H8K in the context of grade 0 rejection vs. grade 3-4 rejection.

Pathways	Distribution (%)
Regulation of immune response (IR)	26.52
Innate immune response	26.50
Positive regulation of IR	21.5
Adaptive IR	10.36
Humoral IR	7.59
Cell activation involved in IR	5.0
Negative regulation	2.53

RESULTS

Our study had two folds: first to review the literature within the last 5-6 years (2016-2022), mainly with the goal of non-invasive biomarkers associated with kidney transplant outcome. We selected studies that utilized various sample types, either peripheral blood or urine and molecular diagnostic methods for prediction of allograft outcomes (Table 1.). Second to use database of our own studies of genomic analysis of biomarkers mainly represented by gene expression signatures and correlated with clinical outcomes at post-transplantation. In general, most biomarkers either had an immunologic basis, known to be associated with irregular immune response or were non-immunologic biomarkers, such as HMGB1 (high-mobility group box-1), heat shock proteins (HSPs), cell debris etc., those released from injured cells, and has potential for activating the innate immune system, leading to a wide range of pathophysiologic responses. Figure 1., is a graphic presentation of innate and adaptive immunity development due to damage to the transplant, cell maturation/activation, cytokine production, inflammation and leading to a rejection episode.

We observed rapid progress that has been made in the development of new technologies and assays for identification of biomarkers that are non-invasive, rapid and cost effective. In a majority of studies, there was an indication that kidney transplant biopsies are still the gold standard for the diagnosis of acute rejection prior to treatment. Also, there was a general consensus that biopsies are invasive, risky, subject to sampling error, inconvenient and may create complications. A selective summary review of biomarker studies associated with kidney allograft outcome is given in Table 1.

Peripheral blood mononuclear cells (PBMCs) gene expression analysis

Alternative to biopsies, in many studies peripheral blood mononuclear cells (PBMC) were used for gene expression profiling by sequencing, microarray analysis or quantitative polymerase chain reaction (Q-PCR) for accurate diagnosis of post-transplantation clinical outcomes and to avoid the need for a kidney biopsy. Several studies have invested in utility of peripheral blood gene-expression signatures for the diagnosis of subclinical acute rejection after an early stage of transplantation. However, these signature genes in most studies, distinguished between rejections versus no rejections, but were not significantly effective in separating the differences between acute cell-mediated rejection (ACMR) and ABMR (Marx, et al., 2019). Peripheral blood miRNA, including miR-15b; miR-16; miR103a; miR-106a; miR-107 have shown significant improvement in TCMR diagnosis, although future validation is needed since expression measurement in blood cells does not allow identification from ABMR or interstitial fibrosis and tubular atrophy (IF-TA) (Matz et al., 2016;).

Urinary cell mRNA and microRNA

Suthanthiran et al., 2014, in clinical trials of organ transplantation 04 (CTOT-04) utilized urinary-cell mRNA as potential non-invasive diagnostic biomarkers for identification of acute cellular rejection. In large cohort of 485 patients, using urinary cell mRNA, a combination of immunologic

markers, CD3ε, programmed cell death (PD-1), interferon inducible cytokines, CXCL10, CD105, CD14, 18sRNA, TLR4 were elevated and distinguished ACMR, and ABMR from tubular injury. Additional urinary biomarkers derived from metabolic profiling has potential to identify TCMR from stable graft function (Kim et al., 2019;). Urine miRNA profiling have shown 22-miRNA signature associated with allograft failure (Maluf et al., 2014;). These include: miR-142-3P; miR-204; miR-107; miR-211 and miR-32, that have been used to predict recipient's allograft function. However, accuracy of miR requires further validation (Maluf et al., 2014;).

Serum or plasma for detection of biomarkers

Detection of circulating donor-specific antibody (DSA) found in recipients' serum is an early biomarker for diagnosis of antibody mediated acute rejection. Currently, transplanted kidney is monitored through a combination of clinical, (testing for proteinuria); immunologic factors (detection of DSA); Ultrasound-Doppler and histopathology (needle biopsy). The pathogenicity of immunologic factors such as HLA antibodies of DSA depend on characteristics of HLA type, Class I vs. Class II, IgM vs. IgG and c1q complement binding capacity (Yell et al., 2015;). Circulating donor HLA-specific B-cells determined by enzyme-link immunoassay (Karahan t al., 2017;) as well as the recipient's T-cell alloreactivity measured by INF-γ- ELISPOT correlated with both predicted ABMR in and TCMR (Hricik et al., 2015;) in DSA-positive recipients. Identification of markers for Acute ABMR during early post-transplantation is very important and is associated with delayed graft function (DGF) and the development of chronic ABMR.

Single cell utility for prediction of allograft function

Single cells were utilized for distinguishing donor vs. recipient origin and the outcome of allograft status. Donor and recipient immune cell chimerism, could be observed by using cells from peripheral blood or needle biopsy from kidney recipients in a single nucleotide polymorphism (SNP) analysis and in a single-cell RNA sequencing (scRNA-Seq). These are unique studies in which transplant patients are preconditioned prior to receiving the donor bone marrow derived fractionated cells with the goal of maintaining mixed chimerism (mixture of genetically distinct lymphoid cells) for tolerance induction and prolongation of organ survival (Lowsky and Strober, 2022; Malone et al., 2020;). During mixed chimerism the immune system becomes attenuated and trained not to response but the rejection is not completely prevented. The rejection status varied with the ratio of donor/recipient lymphoid cells. Equal ratio of recipient/donor lymphoid cell lineage were observed in good graft function, but not during rejection.

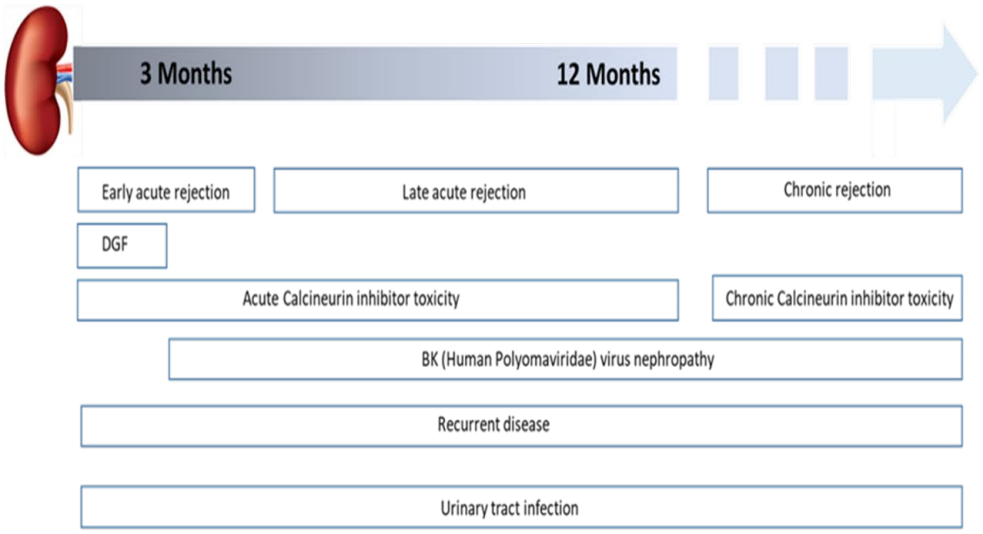
Demographic and clinical characteristics of patients

Four hundred patients who received kidney transplants between 2006 and 2012 were studied. All patients with DGF presented with low GFR (<15 mL/min). Eighty percent of those with low GFR had experienced a rejection episode (RE) or interstitial fibrosis-tubular atrophy (IFTA) and/or kidney infarction. The rejection rates in association with the length of transplantation and clinical outcomes are given in Figure 3. African American (AA) constituted of 76.9% and Caucasians (CAU) 71.9% of the study population. The demographic and clinical variables in the study population is given in Table 1. Patients were assessed based on DGF vs. no DGF and impact of cold ischemia time (CIT). Only 7.6 % (30 out of 390) developed DGF and 13.6% of patients who during the first week post-transplantation (p-RTx) needed dialysis. The CIT was about the same between the two groups. Overall, 66 out of 390 patients experienced episodes of rejection, and twenty-seven (27) recipients returned to dialysis (7%) within 36 months (Table 3.). In addition, as a comparison we given a schematic presentation of the timing of graft -associated complications at different point after transplantation (Figure 2a.) and compared with our data base up to 36 months post transplantation (Figure 2b.).

Table 3. Demographic and Clinical Variables in Study Population

	DGF (N=30)	DGF (N=360)
Age (year)	40.2±13.6	51.50 ±12.5
Gender (F%)	40.0	37.0
Race (AA%)	76.9	71.9
CIT (hour)	19.0±6.8	20.1±8.20
WIT (Minutes)	36.6±21.2	32.5±22.5
1st week dialysis	13.6%	

DGF-delayed graft function
AA-African American
CIT-cold ischemia time
WIT-warm ischemia time



Adapted from: Eikmans M, et al. 2019, Frontiers in Medicine, doi: 10.3389/fmed.2018.00358www.frontiersin.org

Figures 2a. (Figure 2a.) schematic presentation of the timing of graft-associated complications at different point after transplantation. Abbreviations are: M, month; DGF, delayed graft function; CNI, calcineurin inhibitor; BKVN, BK virus nephropathy. Adapted from: Eikmans M, et al. 2019, Frontiers in Medicine, doi: 10.3389/fmed.2018.00358 www.frontiersin.org,

DGF	NonComp	Polycys	Acute Re	Chron Re	Cellular Re	Humoral Re	Cell & AB Re	SGF
30/7.6%	4/1%	2/0.5%	10/2.5	2/0.5%	31/7.9%	2/0.05%	10/2.5	329/84.%

Figure 2b. a graphic demonstration of rejection types and rates vs. the length of RTx survival. Abbreviations: DGF: delayed graft function; RE: rejection; Chron RE: Chronic rejection; SGF: stable graft function; Polycys: polycystic tubular fibrosis; Noncomp: noncompliant; RTx: rental transplantation.

Expression profiling of genes and rejection episodes

We screened microarray analysis by the hierarchical clustering of genes according to the correlation of their expression patterns observed with peripheral blood mononuclear cells (PBMCs) from patients with RE and those with no RE. We tested eight samples of each group with human 19k and 8k gene probe described in the methods. Gene ontology classification showed a majority (24.05%) of genes were associated with an immunological parameter, described in the method. Therefore, we extracted only the gene subset of interest and studied expression levels of innate immune response genes in association with RE/SGF. Functional relationship using web-based DAVID classification tool (http://david.abcc.ncifcrf.gov) (Huang DW, et al, 2007) showed strong positive/closer relationship between AIF-1, TLR-2/TLR-4, IL-18, MY-D88 vs. IL-10. Thus, we tested the level of AIF-1, IL-18, TLR-2, TLR-4, MY-D88, IL-10 as well as IFN-γ in a majority of patients who had undergone kidney allograft transplantation and their PBMCs were available.

Association between Molecular Markers and Clinical Outcomes

Patients specimen were examined using kidney tissue sections and mRNA transcripts. Immunohistochemical (IHC) staining was performed using kidney tissue slices with and without REs and the IHC protocol. AIF-1 and TLR-4 signals presented with a high stain score (HSS) in tubular epithelium, glomeruli and infiltrating mononuclear cells, particularly in samples with grade 3A rejection. The immunoreactivity was moderate in samples with 1A rejection shown in Figure 3a. The IL-18 and TLR-2 signals were observed with minimal immunoreactivity in samples with 1A and 3A rejection, but was observed with an HSS in tubular cells.

The mRNA transcript for quantification of the study markers were normalized to β-Actin. The normalized values (Units) were presented as mean ±SEM. Patients PBMCs were tested at three time points: Pre-transplantation, day 3 and day 6 using a Q-RT-PCR and primers for AIF-1, IL-18, TLRs and MY-D88. The AIF-1 and TLR-2 mRNA transcripts were less

obviously increased in both DGF and in patients who returned to dialysis (RD) comparing the pre-transplant samples vs. Day 3 and Day 6 post-transplantation ($p<0.001$ vs. $p<0.03$ and $p<0.04$ Figure 3b). AIF-1 and TLR-2 expression levels were >2 -fold increase; after transplantation in both DGF and RD groups. The IL-18 transcript expression level was approximately 3.6 -fold increase; on day 6 post-transplantation in patients who later developed rejection and returned to dialysis. The TLR-4 transcript expression was unchanged during the first week after transplantation, indicating that expression levels of AIF-1, IL-18 and TLR-2 transcripts in the peripheral blood could predict the outcome of allograft failure as early as one-week post transplantation.

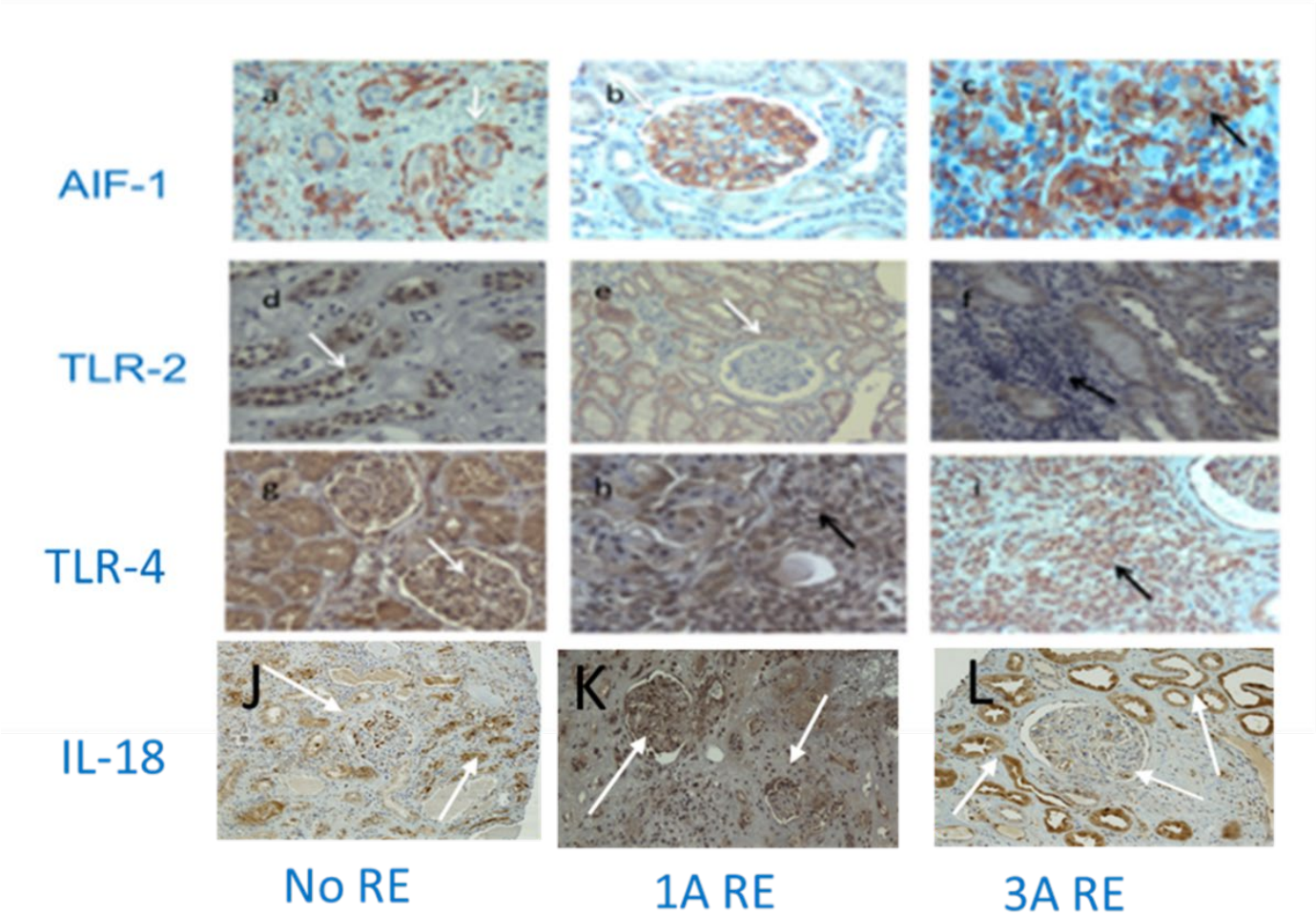


Figure 3a, (A-L) immunohistochemical stain of AIF-1, TLR-2, TLR-4 and IL-18 in association with grades 0-3A rejections. Arrows indicate aggregates associated with rejection.

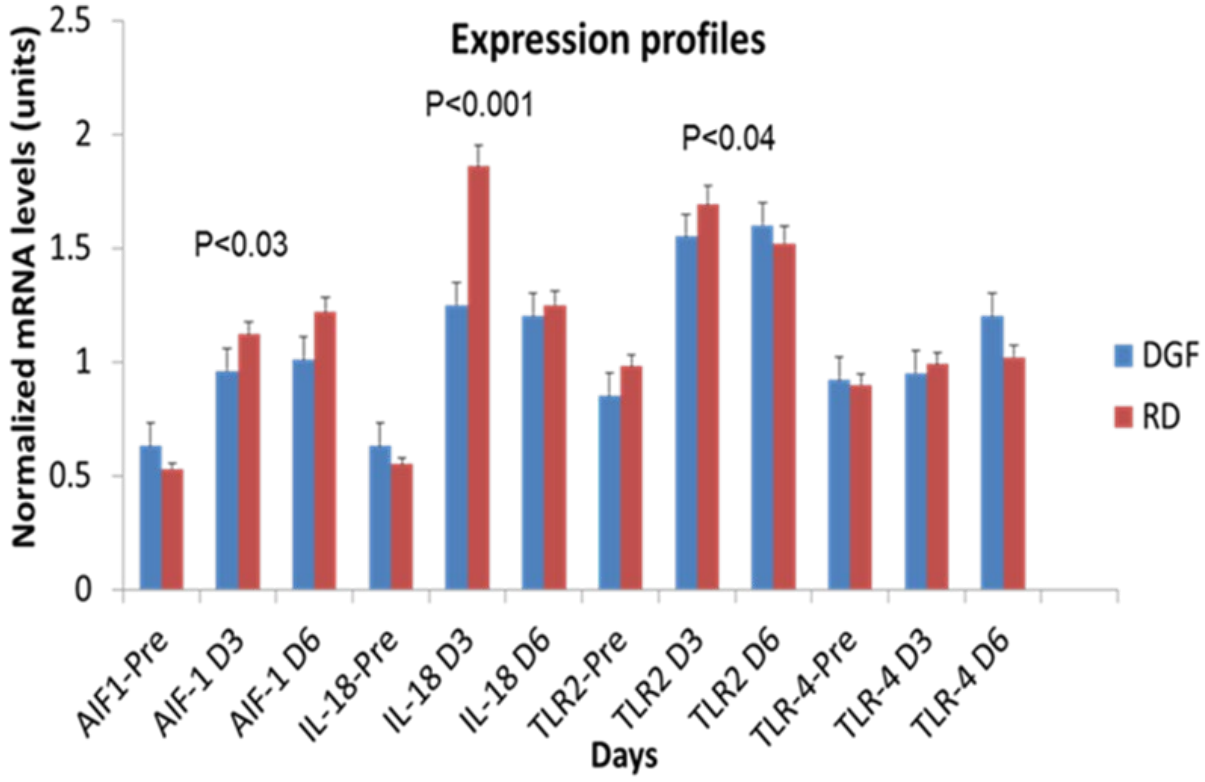


Figure 3B, shows expression profiles of molecular markers on days 3 and 6 post transplantation with CIT≥20 preservation time. AIF-1 and IL-18 increase significantly on day 3 but TLR- 2 and TLR-4 on day 6.

Association with Cold/Warm Preservation-time, Outcome of Kidney Graft Function and Molecular Markers

The impact of cold vs. warm ischemia was tested based on GFR levels. Patients who received a kidney within a CIT≤10 hour had a better clinical outcome based on the eGFR values. These patients experienced GFR values ≥ 55 at an earlier day than those patients who received a kidney within a CIT≥20 hour (Figure 4a), indicating that prolongation of CIT causes a delay in graft function. Furthermore, in a narrower range of CIT, a smaller group of patients (13%) who received a kidney within a CIT≤1-5 had GFR≤35, while greater percentage of patients with CIT≤6-10 had GFR ≥55 (Figure 4b). In this study cold and warm ischemia time inversely impacted the outcome of allograft function. AIF-1 and IL-18 mRNA transcripts increased significantly on Day 3 with CIT between 10-20. TLR-2 and TLR-4 transcript increased significantly with CIT≥20 on days 3 and 6 post-transplantation. Whereas MY-D88 which is an adapter protein for transferring signals from TLRs for early activation of innate immune system was down but, then there was a 3-fold increase on day 3 and 6 regardless of CIT.

Figure 4a. Correlation between CIT and GFR 6 days after RTx

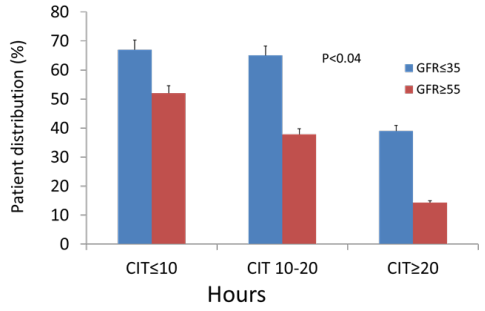


Figure 4b. Correlation between a narrow range of CIT and GFR 6 days after RTx

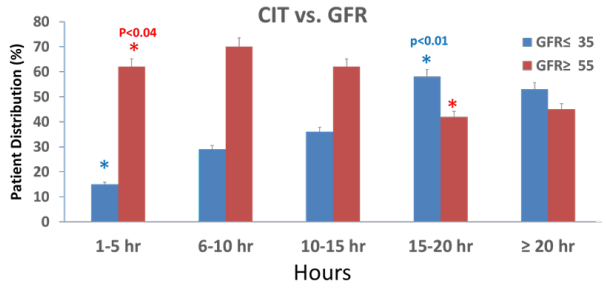


Figure 4c. Expression levels of AIF-1, IL-18, TLR-2, TLR-4 and MYD88 during CIT

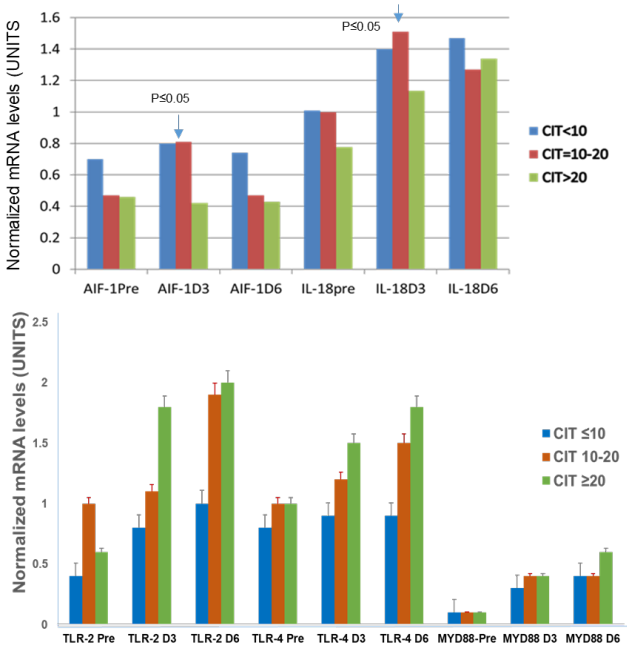


Figure 4. Correlation between cold ischemia time and outcome of transplant. The left upper panel (Figure 4a) presents correlation between cold ischemia time and GFR six days after transplantation. Patients who received a kidney within a CIT≤10 hours had a better clinical outcome based on the eGFR values. The left lower panel (Figure 4b) shows correlation between a narrow range of CIT and GFR 6 days after transplantation. Smaller percentage (13%) of patients who received a kidney within a CIT ≤1-5 hours had GFR≤35 while greater percentage of patients with CIT≤6-10 hours had GFR ≥55. The right panels (Figure 4c) presents mRNA expression levels of molecular markers. AIF-1 and IL-18 mRNA transcript increased on day 3 with CIT between 10-20 but TLR-2 and TLR-4 transcripts increased significantly with CIT≥20 on days 3 and 6.

DISCUSSION

With the advances in innovative technology, new biomarkers for prediction of clinical outcomes after kidney transplantation are considered the potential basis of precision medicine. However, such biomarkers for application in medical practice require validation and proof through multi-cohort studies. In the field of transplantation, non-invasive biomarkers are ideal for sequential screening of allograft status for determination of clinical outcomes and patient's safety. For such reasons, a wide range of biomarkers have been tested and verified in biological fluids including peripheral blood (lymphocytes, serum and plasma) as well as in urine specimen (See Table 1.). Biological fluids sometimes referred as to liquid biopsy. Major advantage of the use of liquid biopsy over the traditional renal biopsy, is cost effectiveness, convenience, non-invasiveness, less sampling errors and patient safety. Biomarkers are ideal for early determination of allograft status, including delayed graft function and acute rejection episodes (Maier et al., 2018; Eikmans et al., 2019;). Quaglia et al., 2020; characterized the biomarkers in seven different types and their role in identification/estimation of clinical status of allograft transplant. These include: susceptibility or risk; diagnostic; prognostic; predictive; monitoring; pharmacodynamics/response and safety biomarkers. A perfect biomarker more likely, must have high sensitivity and specificity with a positive predictive value for determination of subclinical status of allograft and monitoring therapeutic response. Traditional non-invasive monitoring of newly transplanted kidney, include sequential measurements of serum creatinine for occurrence of proteinuria; estimation of glomerular filtration rates (eGFR), and assessment of donor

specific antibodies (DSA) for early diagnosis of antibody mediated rejection (AMR) which are significantly relevant to the allograft survival and therapeutic decisions. However, these conventional methods are less sensitive, and urges utility of molecular biomarkers for a better implementation and detection of immunological risks factors involve in allograft transplantation.

Through the high-throughput technologies in genomics, proteomics and metabolomics scientists have discovered many candidate biomarkers that can be measured in the peripheral blood and urine specimen as described earlier. Of the thousands of high-quality biomarkers only two transplant biomarkers, the Allomap (Deng et al., 2006;), and the Immuknow, Eurofins (Sottong et al., 2000;) were approved by the FDA. The Allomap, constituted of a panel of 11- genes in peripheral blood to distinguish acute rejection from no rejection. Immuknow assay measures ATP released by the CD4 lymphocytes, found to be correlated with prediction of rejection episodes. Myslik et al., 2014, suggested that the Immuknow assay is useful for the development of personalized protocol for immunotherapy after kidney transplantation and monitoring rejection episodes.

Additional molecular biomarker assays are in the market and recently allowed coverage through Medicare and Medicaid Services. The AlloSure assay (CareDx, Inc, Brisbane, CA) has been validated for identification of early stages of rejection episodes in kidney transplant recipients. The test utilized circulating donor-derived cell free DNA (dd-cfDNA) in the blood (Bloom et al., 2017). It has been shown in cohort studies that the dd-cfDNA levels of more than 1% in blood circulation was associated with active TCMR (rejection grade: ≥1B) (Thongprayoon et al., 2020, Fillipone and Farber 2021). Although false elevated levels of dd-cfDNA in some recipients observed due to the medical conditions or ageing, suggest serial testing may be required.

The kSORT (Kidney Solid Organ Response Test) is another immune assay, representing 17-gene set associated with acute allograft rejection. The assay has 100% sensitivity and has greater predictive value over current biopsy test (Roedder et al., 2014). Another assay derived from Genomics of Chronic Allograft Rejection (GoCAR) is a microarray-based, 13-gene set, predicted kidney fibrosis at one year post transplantation and early graft loss (O'Connell et al., 2016, Friedewald et al., 2019), but it is not clear whether this test can discriminate between interstitial fibrosis-tubular atrophy and stable graft function.

The TruGraf molecular diagnostic test is also blood driven and well validated test developed by Transplant Genomics Inc. (TGI, Mansfield, Massachusetts, USA). This assay also measures gene expression signatures using a microarray analysis. TruGraft unlike the GoCAR, can predict stable graft function and an immune quiescence, thus, the test may be ideal for monitoring immunosuppressive levels in post-transplant protocols (First et al., 2019).

Clarava and Tuteva (Verici, Dx Inc.) are commercially developed assays, which targeted RNA expression (TREx) and tested in 14 international transplant cohorts. Clarava is a pre-transplant test for prediction of early allograft rejection. Tuteva is a post-transplant test for determination of acute cellular rejection. Both tests are in the process of validation by the study centers (Zhang et al., 2019; and Westphal and Mannon, 2022;). In fact, the finalized data was presented at the American Transplant Congress in June 2022, indicated that these tests have a stronger positive predictive value (PPV), compared with currently available non-invasive test.

Urinary cell mRNA, described earlier has shown great potential as a non-invasive diagnostic assay for detection of acute rejection (Suthanthiran et al., 2014), but the diagnostic accuracy was less sensitive for determination of subclinical rejections. In addition, small quantities of urinary mRNA limit the utility of testing and may not meet the test requirement (Suthanthiran et al., 2014, Guzzi, et al., 2020). Confounding variables such as urinary chemokines or urine NGAL (neutrophil gelatinase-associated lipocalin), released from kidney tubules during nephropathy, stress and inflammation and were proposed as a predictive biomarker for DGF (Hall et al., 2010) will require validation and standardization of the protocols.

Expression profiling approach where thousands of genes analyzed simultaneously to identify cluster of

genes associated with a particular immunologic response and allograft function may determine the fate of allograft transplantation. Evidence supports the possibility that molecular markers such as cytokines and inflammatory mediators play a crucial role in the development of rejection episodes after kidney transplantation. Pre-transplantation allograft conditions such as cold ischemia time or warm ischemia time are major contributing factors to long term survival of the allograft. Identification of such molecular markers may provide a beneficial effect on graft function and allograft survival. In our study we examined expression levels of cytokines, AIF-1, IL-18, TLRs and MY-D88 at early post transplantation in association with cold ischemia time, DGF and GFR as a measure of kidney function with the idea that early changes in inflammatory biomarker production may be predictive of allograft function. Microarray cluster analysis targeted the genes associated with the innate immune response parameters showed in Table 2. There was shown a closer relationship between AIF-1, TLR2, IL-18, IFN-γ, MYD88 and IL-10. Previously we have shown AIF-1, IL-18, IFN-γ and IL-10 mRNA transcript levels in association with GFR. However, IL-18 transcript was increased with GFR in both low and high GFR, while IL-10 mRNA expression was low with low GFR and was threefold increased with high GFR (McDaniel et al., 2013) suggesting, IL-10 as an anti-inflammatory cytokine marker protects the graft from rejection. In contract, AIF-1 mRNA was markedly increased with low GFR, during the time when allograft function is poor, but, showed twofold decrease with high GFR. AIF-1 is an evolutionarily conserved structural protein and is interferon gamma (IFN-γ) inducible protein, expressed by monocytes, macrophages and dendritic cells (Utans et al., 1995 and Zhao et al., 2013), thus, plays an immunomodulatory role in promoting pro-inflammatory responses either toward adaptive immunity or tolerance induction in allograft transplantation. It has been implicated in regulation of inflammation and recently has been shown to be associated with allograft rejection after transplantation (Zhou X, et al., 2011; McDaniel et al., 2013;). Furthermore, AIF-1 mRNA was expressed in podocytes of human kidney glomeruli as well as in urinary sediments of patients with glomerular nephritis (Tsubata et al.,), thus, podocytes damage or failure may account for initiation of renal dysfunction Therefore AIF-1 could be a novel molecular marker in urinary sediment for identification of nephritis and allograft dysfunction after transplantation.

Majority of non-immunologic donor specific factors such as cold ischemia time (CIT) have been associated with the development of DGF (Ditonno et al., 2013, Sert et al., 2014; Helanterä et al., 2020;). In this study, we demonstrated CIT ≥20 hours have a great impact on DGF and subsequent allograft rejection (Figure 4.). Elevated levels of inflammatory molecular markers associated with allograft function observed by eGFR and confirmed by mRNA profiles (Figure 4c) provides a direct association between molecular biomarkers and a role that they may play in prediction of allograft function. An undirected intersection of network gene/protein analysis revealed interactive signals between AIF-1 and multiple immunologic biomarkers including TL-2, IL-18, MyD88 and IL-10 involved in the inflammatory signaling pathway which may uniquely regulate the innate immune responses involved in organ transplantation.

CONCLUSION

The development of and validation of non-invasive biomarkers that measure different biological signals in recipient's samples are needed for accurate prediction of clinical outcomes in allograft recipients at pre and post-transplantation. Accuracy in determination/prediction of early onset may secure long-term allograft survival through contribution of both immunosuppressive therapeutic measures and patient safety.

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