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Review

Systematic scoping review evaluating the potential of wastewater-based epidemiology for monitoring cardiovascular disease and cancer

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HIGHLIGHTS

• Literature analysis identified urinary biomarkers of cardiovascular disease (CVD) and cancer.
• Specificity and risk association assessed between biomarker detection and disease presence.
• Calculated estimates revealed population-level biomarker concentrations in wastewater.
• Five endogenous protein biomarkers deemed feasible for CVD and cancer monitoring by WBE.

GRAPHICAL ABSTRACT

ABSTRACT

Cardiovascular disease (CVD) and cancer are collectively responsible for tens of millions of global deaths each year. These rates are projected to intensify as the COVID-19 pandemic has caused delays in individualized diagnostics, or exacerbated prevalence due to Post Acute Coronavirus (COVID-19) Syndrome. Wastewater-based epidemiology (WBE) has successfully been employed as a useful tool for generating population-level health assessments, and was examined in this systematic scoping literature review to (i) identify endogenous human biomarkers reported to indicate CVD or cancer in clinical practice, (ii) assess specificity to the indicated diseases, (iii) evaluate the utility for estimating population-level disease prevalence in community wastewater, and (iv) contextualize the obtained information for monitoring CVD and cancer presence via WBE. A total of 48 peer-reviewed papers were critically examined identifying five urinary protein biomarkers: cardiac troponin I (cTnI) (heart attack/heart failure), cystatin C (atherosclerosis), normetanephrine (tumor presence), α-fetoprotein (prostate and liver cancer), and microtubule assisted serine/threonine kinase 4 (MAST4) (breast cancer). Next, urinary excretion information was utilized to predict biomarker concentrations extant in community wastewater, resulting in average healthy concentrations ranging from 0.02 to 1159 ng/L, and disease-indicating thresholds from 0.16 to 3041 ng/L. Finally, estimating prevalence-adjusted wastewater measurements was explored in order to assess community-level CVD and cancer presence utilizing U.S. reported prevalence rates. Results obtained suggest that WBE can serve as a viable tool in support of current methods for CVD and cancer assessment to reduce morbidities and mortalities worldwide.

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1. Introduction

For several decades, cardiovascular disease (CVD) has remained the global leading cause of death, with cancer consistently ranked within the top ten (WHO, 2020). These are classified as noncommunicable chronic diseases as their etiology cannot be explained by an acute infectious agent, but are rather influenced by a multitude of genetic, environmental, and/or lifestyle factors and behaviors, with clinical symptoms lasting for extended periods of time (Friedenreich et al., 2021; Islam et al., 2021). If left untreated, the presence of a persistent chronic disease may increase the risk for co-morbidities or other severe complications to arise (CDC, 2021). While both cancer and CVD have a wide range of sub-classifications, their associated risk factors often overlap, such as diet, access to healthcare, or environmental exposures, which has been shown to disproportionately impact certain populations (Bambré et al., 2020). In the United States, these diseases have made a tremendous impact on human life, with historical yearly death estimates reported at approximately 647,000 for CVD and nearly 600,000 for cancer (CDC, 2022b). More recent reports from 2020 indicate these death rates are increasing, likely due to the COVID-19 global pandemic causing significant delays in individualized preventative or diagnostic measures, as well as post-acute COVID-19 syndrome (i.e., "long COVID") leading to several cardiovascular-related co-morbidities (Heron, 2019; Xie et al., 2022).

The process to establish a diagnosis for CVD and cancer typically requires intense and invasive procedures at the individual-level; oftentimes performed by multiple specialists and can include several rounds of biological specimen analyses (i.e., blood, urine, stool), comprehensive imaging, and physical exertion tests (Poirier et al., 2018). These diagnostic procedures can not only become time-consuming and expensive, with annual healthcare costs estimated at approximately $500 billion year^{-1} (USD) for these two diseases combined, but they can also cause a great deal of physical, mental, and emotional strain on the patient and the patient’s support system (Arnett et al., 2019; Poirier et al., 2018; Tarride et al., 2009). Multiple nationwide surveillance programs exist in the U.S. in order to understand population-level trends in risk factor behaviors, exposures, disease incidence, co-morbidities and mortalities, amongst others (CDC, 2021), however, these broad-scale systems rely heavily on self-reported survey data that may be susceptible to recall bias (Pfeiffer et al., 2017). While these various data collection methods are informative, they may either limit insight to only the individuals who have access to healthcare, or contextual and important information is lost due to low-resolution and time-delayed data collection from widescale national surveys (Barrett-Connor, 2011). Ultimately, these limitations to conventional methods for chronic disease data acquisition may necessitate supplementary measurements in order to provide a comprehensive population-level health assessment that encompasses near real-time, contextually-relevant, non-invasive, and inclusive data (Choi et al., 2018).

Wastewater-based epidemiology (WBE) has been successfully implemented for a wide variety of applications, such as monitoring licit and illicit substances (i.e., alcohol, pharmaceuticals, opioids) and, more recently, SARS-CoV-2 during the COVID-19 pandemic (Ahmed et al., 2020; Baz-Lomba et al., 2016; Gracia-Lor et al., 2017; Kitajima et al., 2020). Due to its capability of monitoring community-wide trends of human activity, behavior, and exposure, WBE has been identified as an efficient and cost-effective method for obtaining population-level human health information, while still preserving individual privacy (Kasprzyk-Hordern et al., 2021; Lorenzo and Picó, 2019). Indeed, WBE has been demonstrated in the past to successfully function as an early warning system, such as for poliovirus and hepatitis A and B, that was then later proved to be true once again for SARS-CoV-2/COVID-19 (Böttiger, 1973; Hellmér et al., 2014; Lago et al., 2003; Olesen et al., 2021). There is one report to date that determined feasibility for measuring select anti-cancer therapeutic agents using WBE, suggesting a path forward for future population-scale investigation of cancer (Ferrando-Climent et al., 2016). Several other studies have since either proposed leveraging the benefits of WBE for chronic disease monitoring (Choi et al., 2018; Lorenzo and Picó, 2019; Picó and Barceló, 2021; Vitale et al., 2021), successfully implemented within rural and/or historically marginalized communities where human health information is sparse (Driver et al., 2022a), as well as demonstrated the feasibility of monitoring diagnosed cardiovascular diseases through pharmaceutical measurements (e.g., beta blockers) (Escolà Casas et al., 2021; Rousis et al., 2022). Thus, the methodology not only lends itself as a viable threat-monitoring tool, but also can be applied as a near real-time indicator of intervention efficacy that is inclusive of all community members in order to acquire community-level health information.

As implementation of WBE continues to advance into broader public health areas, it appeared logical to expand on previous work and explore the potential to investigate the utility and feasibility in applying WBE to analyze endogenous, human-excreted, and sewage-bound biomarkers indicative of cardiovascular disease and cancer. Leveraging the degree of morbidity, mortality, and healthcare burden experienced within the United States to serve as a case study, this hypothesized application of WBE was evaluated in order to (i) extract human endogenous biomarkers reported in the literature to indicate CVD and/or
A systematic scoping review of the literature was performed in this study following published guidelines (Peters et al., 2015). Accordingly, literature inclusion and exclusion criteria were established first to inform the scanning and selection of literature appropriate for critically examining the research question of whether WBE has known or potential utility for studying CVD and cancer at the population level.

### 2.1. Systematic scoping literature review

An initial search of the literature was performed using the SCOPUS database for recently reported publications (within 5 years from May 2022). For case-study relevant background information, secondary database searches were limited to the United States. To search for literature that reported on cardiovascular disease (CVD) and individual diagnostic biomarkers, the search terms included “cardiovascular disease” OR “CVD” OR “heart disease” AND “biomarkers” OR “diagnostic” OR “endogenous” OR “human”. Search terms for cancer and individual diagnostic biomarkers included: “cancer” OR “breast cancer” OR “liver cancer” OR “prostate cancer” AND “biomarkers” OR “diagnostic” OR “endogenous”.

### 2.2. Inclusion and exclusion criteria

Studies were excluded if there was no report of urinary or fecal biomarkers, if animal models were used as proxy to humans, and if there were no associations found between the biomarkers under investigation and the diseases of interest. From these searches, papers were then cross-examined to identify studies that reported hazard ratios (HR), or an equivalent metric, to evaluate associations and specificity between identified biomarkers and either CVD or cancer and relevant disease subsets, and if there were significant differences in urinary concentrations between patients and controls. Studies that reported hazard ratios as null (HR = 1) were excluded, while studies reporting low (HR < 1) or strong (HR > 1) association to either CVD or cancer were included for further evaluation. Studies were also excluded if only creatinine-normalized values were reported for urinary concentration of select biomarkers.

### 2.2.3. Calculated ratios and population-level biomarker concentrations

Ratios were calculated for each biomarker to preliminarily evaluate the degree of difference between diseased and healthy individuals using literature reported urinary concentrations (ng/mL) following Eq. (1):

\[
\text{Disease to Healthy Ratio} = \frac{D}{H}
\]

where \( D \) is the literature reported urinary concentration (ng/mL) that indicates disease and \( H \) is the literature reported urinary concentration (ng/mL) of healthy individuals based on clinical investigations. This calculation was repeated for each biomarker of interest for both CVD and cancer and affiliated subset morbidities.

Estimated population-level biomarker concentrations (ng/L) measured in municipal wastewater were calculated utilizing literature reported values in human urine and following Eq. (2):

\[
\frac{\text{IE} \times \text{HU} \times \text{pop}}{F} \times 1,000 = \frac{\text{ng}}{\text{L}} \text{ in wastewater}
\]

where \( \text{IE} \) is the literature reported biomarker value (ng/mL) in human urine at the individual level. \( \text{HU} \) is the average number of liters an individual excretes urine per day (1.4 L) (Medline, 2022). \( \text{pop} \) and \( F \) represent predefined theoretical values for population and flow rate utilized for all calculations in this exercise. Here, a population size of 10,000 contributing individuals with a 0.9 million gallons per day (MGD) flow rate of wastewater were assumed to accomplish this. Conversion from MGD to L/day was used following Eq. (3):

\[
\frac{\text{L}}{\text{day}} = \text{flow (MGD)} \times M \times C
\]

where \( \text{flow (MGD)} \) is the reported flow rate at time of sample collection (commonly as million gallons per day) multiplied by \( M \) (1,000,000) to convert from million gallons per day to gallons per day. \( C \) is the conversion factor from gallons to liters (3.785).

In order to more accurately reflect and estimate disease presence within a given community, prevalence rates were incorporated into the calculations for each identified biomarker in this study. Prevalence-adjusted rates in wastewater (ng/L) were calculated using Eq. (4):

\[
\text{WC (ng/L)} = \text{PR}
\]

where \( \text{WC (ng/L)} \) refers to wastewater concentration (the output from Eq. (2)) and \( \text{PR} \) is the most recently reported estimated prevalence rate of CVD (7.2 %) (CDC, 2022a) and cancer (5.1 %) (NCI, 2020a) in the United States. The result of this equation provides an estimated assessment of whether disease presence could be assessed by wastewater-based epidemiology given a predefined population of 10,000 contributing individuals and a flow rate of 0.9 MGD.

### 3. Results

Here, we performed a systematic scoping literature review to assess the feasibility of performing wastewater-based epidemiology for the purpose of monitoring cardiovascular disease and cancer at population-scale. These literature-reported values were transformed into a theoretical WBE case study that first investigated the current landscape of CVD and cancer in the United States, followed by a series of calculations that could serve to inform future experimental investigation in community wastewater.

#### 3.1. Systematic scoping literature analysis results

A total of 48 peer-reviewed papers were critically examined as a result of this analysis that offered insightful elements on disease etiology, epidemiological information, biochemical pathways and reactions, biomarker discovery, and clinical investigations. All studies identified biomarker detections in either blood (serum or plasma) or urine; no studies indicated fecal excretion as a dominant or viable excretion route. From those reported detections, five urinary protein biomarkers were reported as indicative of specific subsets of cancer and cancer: heart attack/heart failure, atherosclerosis, cancer of the liver, prostate, and breast, and tumor presence (Table 1).

#### 3.2. Assessment of cardiovascular disease and cancer burden in the United States

Cardiovascular disease (CVD) has consistently been the leading cause of death in the United States since 1910 (Mensah et al., 2019). Common cardiovascular-related conditions include peripheral arterial disease, congestive heart failure, myocardial infarction (heart attack), arrhythmias, and stroke (Mc Namara et al., 2019; Mensah et al., 2019). As of 2016, an estimated 121 million people in the United States are suffering from some form of CVD-related morbidity; heart attacks being the most common affecting nearly 805,000 people each year (Fig. 1) (Benjamin et al., 2019). Risk factors for the development of CVD are vast, including individual behaviors (tobacco use, poor diet, high alcohol consumption), family history, genetic, and environmental factors (Burroughs Peña and Rollins, 2017; Johansson et al., 2021; Roth et al., 2020). This raises the importance of adopting routine preventative and comprehensive screening in order to thwart a sudden and potentially fatal cardiac event (Rippe, 2019).
Several of these conditions can quickly become fatal if this buildup of vascular plaque is not detected early, highlighting the importance in support of regular screening methods.

Cancer has remained the second leading cause of death in the United States since 2016, with an estimated 599,108 lives lost per year (Heron, 2019). As of January 2019, approximately 16.8 million Americans are living with cancer, and an additional 1.9 million new cases are estimated to be diagnosed in 2022 (ACS, 2022a). Some of the most common types of cancer in the United States include cancer of the breast, prostate, and liver (Fig. 1)(NCI, 2020a). Cancer is commonly referred to as a silent disease as many of the more noticeable symptoms do not arise until the later stages in progression, in some cases when it’s too late for treatment, or the symptoms are non-specific that could potentially be explained by another less severe condition (Al-Azri, 2016). Evidence suggests the risk of developing cancer is much greater in individuals who have weakened immune systems due to age, underlying conditions, or chronic stress, with socioeconomic status serving as a major driving factor for incidence as well as diminished survival rate due to late diagnosis (Coughlin, 2019). These circumstances can make it nearly impossible to effectively and time-efficiently diagnose and treat an individual thought to have cancer (Coughlin, 2019; Roy and Saikia, 2016). Thus, similar to CVD, continuous screening events that allow for early detection of disease play an essential role in mitigating further progression and effectively treating many types of cancer (Loud and Murphy, 2017).

3.3. Identified biomarkers and association analysis to disease

As mentioned, diagnosing cardiovascular disease and cancer can become a time-consuming and expensive endeavor, however, recent clinical advances that have identified biomarkers excreted in urine hold great promise for proposing investigation via WBE. These biomarkers for population-level detection and assessment for CVD include high-sensitivity cardiac troponin I (cTnI), indicative of heart attack and heart failure, and cystatin C, which has been reported to serve as an indicator for atherosclerosis (Table 1)(Aydin et al., 2019; Felker et al., 2012; Ho et al., 2018; Pervan et al., 2017; Pervan et al., 2018). A common metric to New advances in diagnostic medicine have determined less-invasive urinary measurements are feasible for certain biomarkers at the individual level (Röthlisberger and Pedroza-Diaz, 2017). Particular biomarkers of interest for assessing various cardiovascular-related conditions include those that indicate a heart attack as well as atherosclerosis due to their widespread prevalence and association to sudden CVD-related death (Hassan et al., 2011). Atherosclerosis is an inflammatory disease that can lead to the hardening of blood vessels through the accumulation of plaque, oftentimes without patient awareness; increasing the risk for heart attack, stroke, as well as other co-morbidities such as diabetes (Hassan et al., 2021; Sawada, 2021). Several of these conditions can quickly become fatal if this buildup of vascular plaque is not detected early, highlighting the importance in support of regular screening methods.

| Biomarker | Molecular weight (kDa) | Detectable matrix | Disease indicator | Source |
|-----------|-----------------------|-------------------|-------------------|--------|
| Cardiac-Troponin I (cTnI) | 24 | Blood, Urine | Myocardial infarction (heart attack), heart failure | (Ho et al., 2018; Pervan et al., 2018) |
| Cystatin C | 13 | Blood, Urine | Atherosclerosis | (Ho et al., 2018; Satoh-Asahara et al., 2011) |
| α-Fetoprotein | 70 | Blood, Urine | Prostate & liver cancer | (Chen et al., 2011) |
| NMN | <1 | Blood, Urine | Tumor presence (pheochromocytoma) | (Chen et al., 2011; Eisenhofer et al., 2020) |
| MAST4 | 260 | Blood, Urine | Breast Cancer (DCIS) | (Beretov et al., 2015) |
| Normetanephrine; MAST4; Microtubule associated serine/threonine kinase 4; DCIS; Ductal carcinoma in-situ. |

Table 1. Identified endogenous biomarkers proposed for population-level detection of CVD and cancer by WBE.

![Fig. 1](image) The burden of cardiovascular disease and cancer in the United States. (A) Top 10 deadly diseases in the United States in 2020 shown as number of deaths. (B) Annual estimated economic burden of CVD and cancer in billions of U.S. Dollars (CDC, 2022b). (C) Number of new cancer cases per year for breast (striped pink), liver (solid red), and prostate (dotted blue) cancer (ACS, 2022a). (D) Number of heart attacks per year showing first-time attacks (dotted yellow) and two or more (recurring) attacks (striped grey) (CDC, 2022a).
measure strength of association between an exposure and/or event (i.e., a measured biomarker) and a disease under investigation in prospective controlled clinical studies is the hazard ratio (HR) (Toledo, 2018). A study investigating the strength of using cTnI as an indicator of an upcoming cardiovascular event, such as a heart attack, in 1177 individuals exhibited a hazard ratio (HR) of 1.24 (95% CI, 1.17–1.32), suggesting it to be a strong candidate to serve as an early warning signal for a potentially fatal event (Welsh et al., 2019). Another study reported that first-morning urinary cTnI concentrations >0.004 ng/mL were associated with an increased risk of an upcoming (short-term) cardiovascular event compared to controls, reported as an odds ratio (OR), a similar metric to the hazard ratio, of 3.043, 95% CI 1.448–6.391 (p = 0.003) (Chaulin, 2021).

In western societies, such as the United States, atherosclerosis is the primary cause of heart disease and stroke, and is responsible for approximately 50% of all deaths (Lusis, 2000). In a prospective study of 1004 patients investigating CVD in patients with and without atherosclerosis, cystatin C was significantly (p < 0.001) associated with predicting a future cardiovascular event in those with atherosclerotic plaque or those who developed plaque over time; HR 1.94 (95% CI, 1.31 to 2.88) (Hoke et al., 2010). Human clinical urinary threshold concentrations that would suggest an upcoming or current occurrence of a cardiovascular event or buildup of plaque at the individual-level have been reported as 0.04 ng/mL (cTnI) and 280 ng/mL (cystatin C), compared to reported typical urinary concentration ranges in healthy individuals of 0.01–0.03 ng/mL for cTnI and 52–140 ng/mL for cystatin C (Hassan et al., 2021; Pervan et al., 2018; Welsh et al., 2019; Xiao et al., 2015) (Table 2).

Cancer is another complex and prevalent disease that could be mitigated through early detection and screening efforts, particularly at population-scale. Recent studies have identified a few promising biomarkers for this purpose, including α-fetoprotein, predominately for the detection of prostate and liver cancer, normetanephrine for identifying tumors such as pheochromocytoma, and microbulate assisted serine/threonine kinase 4 (MAST4), for the detection of breast cancer (Table 1) (Beretov et al., 2015; Chen et al., 2011). Breast cancer is the most common type of cancer in women in the United States, constituting about 30% of all new female cancer cases per year (ACS, 2022b). One study that compared the urinary proteome using a semi-quantitative, label-free LC-MS/MS approach between patients with breast cancer and healthy women controls found that MAST4 was up-regulated by 4.2-fold in breast cancer patients, indicating it could be used as a strong biomarker for regular screening at the individual-level (Beretov et al., 2015) (Table 2). Hepatocellular carcinoma (HCC) is one of the most invasive types of cancer in humans, and remains to be the third leading cause of death due to cancer across the globe, while prostate cancer affects approximately 1 in 8 men throughout their lifetime (Zhang et al., 2020). The biomarker α-fetoprotein is commonly used for the detection, prognosis, and management of both prostate and liver cancer, with recent studies reporting elevated urinary levels exhibited in HCC patients compared to controls (HR 1.55; 95% CI 1.3 to 1.8) (ACS, 2022c; Hsu et al., 2015; Pan et al., 2020).

Normetanephrine is produced by the action of a catechol-O-methyltransferase enzyme from norepinephrine that is excreted in urine, and is commonly used as a marker for tumors such as pheochromocytoma; a neuroendocrine tumor that can lead to potentially fatal comorbidities including hypertension, chronic headaches, and damage to various organs such as the liver and adrenal glands (NCI, 2020b; Plouin and Gimenez-Roqueplo, 2006). While pheochromocytomas are often considered rare, it is estimated that current prevalence rates are vastly underreported due to their nonspecific presentation of symptoms (NCI, 2020b). Nonetheless, normetanephrine has been indicated to be a strong indicator for pheochromocytoma, with increased measures in plasma as high as 36-fold when compared to healthy controls (Gupta et al., 2015). Studies reporting urinary thresholds that can indicate potential presence of cancer at the individual level are 0.03 ng/mL and 740 ng/mL for α-fetoprotein and normetanephrine, respectively, compared to healthy urinary concentration ranges reported as non-detect (ND) to 0.01 ng/mL for α-fetoprotein, and 34–530 ng/mL for normetanephrine (Table 2) (UCLA, 2022; Zhan et al., 2020).

3.4. Estimated population-level biomarker concentrations in wastewater

While the ratios are informative to provide insight on the difference in up-regulation in diseased individuals, it was necessary to translate the literature reported urinary concentrations for each biomarker into a theoretical exercise based on real-world values for detection in community wastewater. Calculations were performed assuming the scenario of investigating within a relatively small wastewater catchment at neighborhood-scale comprised of a population of 10,000 contributing individuals resulting in approximately 0.9 million gallons per day (MGD) of wastewater flow. This analysis revealed that all identified biomarkers, with the exception of MAST4 which has only been reported thus far as a presence/absence biomarker, resulted in concentrations that could theoretically be monitored in wastewater based on these assumptions (ng/L): cTnI (0.04–0.12), cystatin C (214–575), α-fetoprotein (ND-0.04), and normetanephrine (140–2178) indicating healthy ranges, with disease-indicating measurements estimated to be (ng/L): cTnI (0.16), cystatin C (1,150), α-fetoprotein (0.12), and normetanephrine (3041) (Table 3). Finally, since it is unlikely for all individuals of any given community to be afflicted with CVD and/or cancer, prevalence rates for CVD (7.2%) (CDC, 2022a) and cancer (5.1%) (NCI, 2020a) were incorporated to adjust for disease prevalence within this simulated population size of 10,000 individuals as it is relevant to the United States (ng/L): cTnI (0.01), cystatin C (83), α-fetoprotein (0.01), and normetanephrine (155) (Table 3). Based on these prevalence-adjusted population-level concentrations, the strongest biomarkers that present with the most promise to indicate disease presence using WBE are normetanephrine and cystatin C, followed by cTnI and α-fetoprotein.

Table 2

| Biomarker        | Healthy reference ranges (ng/mL) | Reported urinary disease thresholds (ng/mL) | Source                                                                 |
|------------------|----------------------------------|---------------------------------------------|-----------------------------------------------------------------------|
| Cardiac-Troponin I (cTnI) | 0.01–0.03                        | 0.04                                        | (Pervan et al., 2018; Welsh et al., 2019)                               |
| Cystatin C       | 52–140                           | 280                                         | (Conti et al., 2006; Hassan et al., 2021; Herget-Rosenthal et al., 2004) |
| α-Fetoprotein    | ND–0.01                          | 0.03                                        | (Mor et al., 2015; Zhan et al., 2020)                                  |
| NMN              | 34–510                           | 740                                         | (UCLA, 2022; Unger et al., 2006)                                      |
| MAST4            | N/A                              | 4.2-fold*                                   | (Beretov et al., 2015)                                                |

* NMN: Normetanephrine; MAST4: Microtubule associated serine/threonine kinase 4; ND: Non-detect; N/A: Not applicable.

* MAST4 was found to be upregulated by 4.2-fold in breast cancer patients compared to healthy controls.
Normetanephrine and cystatin C represent both CVD and cancer-related morbidities, such as tumor presence and atherosclerosis, thus, incorporating WBE may serve to enhance current public health monitoring efforts.

4. Discussion

While biomarker discovery and medical detection of diseases such as CVD and cancer are expanding, prevalence of these diseases continue to exhaust the efforts of medical and public health professionals. Promptness in detection and monitoring are key for improving global health. Compared to clinical investigation, WBE is often viewed as a rapid and cost-effective method to continue to pursue specific and human-excreted biomarkers. When performing WBE both traditionally and in its more current state, untreated wastewater samples consisting of composites representing contributions (i.e., urine, blood, feces, sputum) from human populations are collected at a wastewater treatment plant (WWTP) (city-level), from within the sewer collection system (neighborhood-level), or in a smaller community setting, such as a university campus (near-source or building-level) (Bivins et al., 2022; Choi et al., 2018; Spurbeck et al., 2021). Sample collection from within the sewer infrastructure or at the building-level would likely serve to enhance detectability of these human-excreted endogenous compounds given their potential vulnerability to in-sewer degradation (Bowes et al., 2022; Hart and Halden, 2020). Comparing measurements of endogenous biomarkers to other compounds that are associated with these diseases, such as pharmaceuticals typically prescribed for treatment of various CVDs including statins, beta-blockers, diuretics, or antihypertensives, as well as risk factors for contributing to disease including smoking, alcohol use, or poor dietary behaviors, may also be of interest to further crystallize downstream data interpretation; with several of these already investigated by WBE practitioners (Choi et al., 2020; Galani et al., 2021; Röthlisberger and Pedroza-Díaz, 2017). For cancer, this could also be applied through measurements of chemotherapy or other therapeutic intervention drugs as 75% of the procedures are outpatient, thus the human excreted markers would likely be contributed to the local wastewater collection system (Kosiej and Heath, 2011). For the identified biomarkers that have been shown to peak during the first-morning urine, such as elevated cTnI that can indicate an upcoming cardiovascular event, this could also inform new stratagical approaches for time-targeted wastewater sample collection in order to enhance signal detection while also providing near real-time, actionable data. Further, instrumentation commonly used for chemical analysis by WBE to understand community-level metabolomics has largely involved liquid chromatography-tandem mass spectrometry (LC-MS/MS), while biological analyses has leveraged genomic approaches including quantitative PCR (qPCR, RT-qPCR) or sequencing (shotgun, next generation, etc.) in response to the COVID-19 pandemic (Ahmed et al., 2020; Gracia-Lor et al., 2017; Zuccato et al., 2008). A new frontier for WBE is the recruitment of proteomics in order to continue to advance the field and inform on other unique aspects of human health, behavior, exposure, and activity. Few studies have begun exploring this novel avenue which will serve as a foundation to inform future work; including the detection of the herein proposed CVD and cancer protein biomarkers that include a wide range of molecular weights (kDa) (Table 1) (Carrascal et al., 2020; Lara-Jacobo et al., 2022)

As many WBE studies are commonly performed on a longitudinal basis, with sample collection occurring at a regular cadence, this method can ultimately serve to complement the traditional medical model of ‘grab sampling’ individual patients, as these time-discrete samples only reflect the health status at one point in time from a single person. Thus, a great benefit to WBE is the ability to exhibit trends over time of all individuals within a given community, not just those who are able to seek regular health screenings by a physician, offering unique insights that would then prompt relevant public health interventions when necessary; ultimately adopting a diagnostic approach for precision public health. The results presented herein demonstrate the feasibility for longitudinal monitoring within a relatively small wastewater catchment at neighborhood-scale, with the

Table 3

| Biomarker          | Disease indicator | Estimated population-level biomarker concentrations in wastewater | Reported U.S. prevalence rates | Source                      |
|--------------------|-------------------|----------------------------------------------------------------|------------------------------|-----------------------------|
|                    |                   | Healthy range (average) (ng/L) | Disease indicating (ng/L) | Prevalence-adjusted (ng/L) |                           |
| Cardiac-Troponin I (cTnI) | CVD               | 0.04-0.12 (0.08) | 0.16 | 0.01 | 7.2% (CDC, 2022a)     |
| Cystatin C         |                   | 214-575 (394)   | 1150 | 83  |                                |
| a-Fetoprotein      | Cancer            | 60-0.04 (0.02)  | 0.12 | 0.01 | 5.1% (NCI, 2020a)        |
| NMN                |                   | 140-2178 (1159) | 3941 | 155 |                                |

NMN: Normetanephrine.

These bene...
calculated prevalence-adjusted disease rates based on reported U.S. estimates ranging from 0.01 to 155 ng/L within a community of 10,000 contributing individuals (Table 3); indicating this type of analysis shows promise to support conventional methods. The purpose of incorporating prevalence rates into these theoretical wastewater-derived estimations was to highlight the contextual and inclusive nature of WBE as these rates may differ across diverse communities. It is evident that CVD and cancer afflict certain subsets of populations disproportionately (Burroughs Peña and Rollins, 2017), demonstrating the need for measurements that are inclusive of the entire population served. Thus, incorporating reported prevalence rates at the level of the investigators’ respective municipality, city, or state, where applicable, in downstream WBE data analysis to triangulate the collected wastewater-derived data may serve to accomplish this endeavor.

Finally, more research investigating long-term impacts of COVID-19 support strong connections between post-acute COVID-19 syndrome and increased risk of incident cardiovascular events, such as stroke (hazard ratio (HR) 1.52; 95 % CI 1.43, 1.62), heart failure (hazard ratio (HR) 1.72; 95 % CI 1.65, 1.80), and dysrhythmias (hazard ratio (HR) 1.69; 95 % CI 1.64, 1.75) (Xie et al., 2022). Thus, it is not only timely but imperative to begin investigating the use of WBE to understand these trends in CVD-related events in order to prospectively and proactively monitor long-term and significant burden as the pandemic continues.

4.1. Potential limitations and guidance for future work

While WBE has been identified here to potentially represent a powerful tool in the tracking and monitoring of global deadly diseases, there are certain limitations that should be considered. For example, endogenous biomarkers are commonly excreted in much lower concentrations, rendering detection and confident quantification more challenging. Mixing of urine with stool also injects uncertainty as to the absolute quantity of biomarkers present and makes the detection of urinary biomarkers more difficult due to increased matrix effects of fecal matter. Furthermore, biomarkers may be fairly stable in urine but much more susceptible to degradation or transformation within the sewerage system, due to the presence of fecal microbiomes and active biofilms covering the inner linings of sewer pipes. Attempting to circumvent this issue by collecting samples closer to the point of contribution may enhance detection abilities, but simultaneously may raise ethical concerns due to the possibility of potential identification and stigmatization of affected sub-populations or even individuals (Coffman et al., 2021; Jacobs et al., 2021). Stability tests investigating degradation rates are warranted to build on this literature-analysis for future experimental investigation to help inform study design and facilitate the interpretation of results obtained, in addition to understanding interactions with other constituents and characteristics of wastewater, and how that may impact detection signal (pH, hydrophobicity, etc.). To the author’s knowledge, this type of relevant information needed for a WBE study is lacking.

The specificity of biomarkers for particular diseases also needs to be considered, as some may be indicative of more than one type of disease and associated morbidities. For example, elevated levels of a-fetoprotein can indicate either prostate or liver cancer, while cystatin C has also been shown to increase in patients with renal failure secondary to atherosclerosis; thus, it is important to note that while these proposed biomarkers would indicate CVD and cancer prevalence overall, identifying specific types or subsets may require more sophisticated techniques for downstream data analysis at population-scale (Conti et al., 2006; Hassan et al., 2021). As mentioned, compounding wastewater-derived data of endogenous markers with measurements of related pharmaceuticals or relevant external data sets, when appropriate, may serve to overcome some of these challenges, and provide a holistic scope of valuable information.

5. Conclusion

Chronic diseases such as CVD and cancer can cause an extreme level of mental, physical, financial, and emotional stress, not only on those affected but also on their families and caregivers. Utilizing WBE to observe population health status in near real-time has been shown to be possible for various health threats, and are indicated to be of value for addressing CVD and cancer through these five urinary biomarkers identified from this in-depth, systematic scoping literature analysis: cardiac troponin I, cystatin C, α-fetoprotein, normetanephrine, and MAST4. In comparison to clinical investigation, this approach may provide a cost-effective, non-invasive, and inclusive method of collecting substantial amounts of population-level data, all while preserving community anonymity. While the aforementioned conventional clinical evaluations provide precise and pertinent information about specific individuals, WBE can be used in concert to offer a comprehensive view of the disease burden in populations on a more inclusive spectrum; those that are disease inflicted yet asymptomatic, and those without access to proper healthcare. Further studies are
warranted and encouraged to confirm full-scale implementation of these indicators of the top deadly diseases across the globe.

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CRediT authorship contribution statement
Vivek Amin: Conceptualization, Investigation, Data curation, Visualization, Methodology, Writing – original draft. Devin A. Bowes: Conceptualization, Investigation, Data curation, Formal analysis, Methodology, Visualization, Resources, Writing – original draft. Rolf U. Halden: Conceptualization, Funding acquisition, Resources, Supervision, Writing – review & editing.

Data availability
Data will be made available on request.

Declaration of competing interest
RUH is a managing member of AquaVitas, LLC and founder of the ASU Foundation non-profit project OneWaterOneHealth operating in the same intellectual space.

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