Biofluid biomarkers in Huntington's disease

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Summary

Huntington's disease (HD) is a chronic progressive neurodegenerative condition where new markers of disease progression are needed. So far no disease modifying intervention has been found and few have been proven to alleviate symptoms. This may be partially explained by the lack of reliable indicators of disease severity, progression and phenotype.

Biofluid biomarkers may bring advantages in addition to clinical measures, such as reliability, reproducibility, price, accuracy and direct quantification of pathobiological processes at the molecular level; and in addition to empowering clinical trials, they have the potential to generate useful hypotheses for new drug development.

In this chapter we review biofluid biomarker reports in HD, emphasising those we likely to be closest to clinical applicability.

Key Words: Huntington's Disease, Biomarkers, Cerebrospinal Fluid, Blood, Urine, Review.

1. Introduction

So far almost 100 clinical trials have been conducted in Huntington's disease (HD), with a very low success rate[1], and there is only low quality evidence that selected symptomatic interventions have a beneficial effect on HD[2,3], while evidence of disease modification has not yet been reported[4,5]. Two possible explanations exist for this: either the drugs did not work, or they worked and we were unable to detect the benefit. Newer therapeutics, like antisense drugs that aim to reduce production of mutant huntingtin, are currently being tested in humans[6], and it is crucial that we are able to detect target engagement and a therapeutic effect if one is being exerted. This is a problem across most neurodegenerative diseases but particularly difficult in HD, where premanifest mutation carriers usually feel and appear completely well for several decades before symptoms begin, and the rate of neuronal death is very slow[7]. Moreover, if such disease-modifying drugs are developed, we will need to know when to initiate treatment and how monitor their effects.

Concerted international collaborative efforts have established a panel of clinical, cognitive and neuroimaging biomarkers with predictive power for onset and progression in HD that can be used to stratify, enrich and conduct clinical trials[8,9,7]. While these and their successors will certainly be useful, they have limited capacity to report on the biochemical and physiological milieu in the CNS, and especially to do so within the timescales necessary to optimise the conduct of clinical trials. In particular, early phase trials and critical go/no-go decisions are greatly facilitated by pharmacodynamic markers to indicate whether or not a drug has engaged with its target to produce an early, meaningful biological effect.

Biofluid biomarkers are so-called because they are quantified in body fluids, ideally with minimal invasiveness, good accuracy, and high discriminatory power. They cover not only the most commonly used fluids such as blood and urine, but also cerebrospinal fluid (CSF), saliva, sweat, among others. Biofluid biomarkers have the appeal of being capable of precise, reliable quantification, often in bulk or in retrospect. In addition, a single sample can generate results for multiple analytes of interest.

CSF is enriched for CNS-derived substances and its collection for research purposes by lumbar puncture is safe and well-tolerated. In other neurodegenerative conditions, CSF has been extensively studied to yield biomarkers that can be used for diagnosis, prognosis and clinical trial conduct but in HD, large systematic studies of CSF are lacking and few findings have been replicated[10]. In blood and more accessible tissues, even less systematic work has been done.

In this chapter we provide a review of proposed biofluid biomarkers for HD, the methodologies used to identify them, and their state of validation. We present the comprehensive details of methods and findings in tabular format, and in the text particular focus has been given to biomarkers likely to be used in current and planned clinical trials.

2. Methods

All studies published after 1993 – the date of *HTT* gene discovery[11] – on HD fluid biomarkers were included (see Fig. 1). The references were retrieved from MEDLINE using the terms “huntington” and (“cerebrospinal fluid” or “blood” or “urine”). Reference lists were cross-checked. No language, or quality restriction was applied. For biomarkers of particular interest, the date range was extended before 1993. The search strategies employed can be found below: (("Cerebrospinal Fluid"[Mesh] OR CSF[Title/Abstract]) AND ("Huntington Disease"[Mesh] OR "huntington"[Title/Abstract])); ("Blood"[Title/Abstract] OR "Serum"[Title/Abstract] OR "Plasma"[Title/Abstract]) AND ("Huntington Disease"[Mesh] OR "huntington"[Title/Abstract]); (("Urine"[Mesh] OR urin*[Title/Abstract]) AND ("Huntington Disease"[Mesh] OR "huntington"[Title/Abstract])).

3. Results

3.1. Huntingtin protein

HD is caused by a CAG repeat expansion mutation in the *HTT* gene on chromosome 4[11,12], and the intracellular presence of the mutant huntingtin protein (mHTT) in affected tissues is one of the hallmarks of this condition. Huntingtin protein has an incompletely understood role in normal brains and peripheral tissues[13], but gains toxic properties when large polyglutamine tracts are present in its amino-terminus[14].

As well as the *a priori* importance of studying the pathogenic agent in patients with the disease, drug development programs based on reducing the expression of huntingtin are well underway[6]. Quantifying mHTT with simple, accurate and reproducible methods is of the utmost importance as a pharmacodynamic biomarker of the effect of such treatments (Table 1). This has proven extremely challenging, since soluble mHTT is present in very low concentrations in all accessible biofluids, and is produced ubiquitously such that it is hard to distinguish CNS-derived mHTT from that generated peripherally.

The first detection of soluble mHTT in human biological fluids was achieved in 2009 by Weiss and colleagues, who quantified soluble mHTT in human whole blood, isolated erythrocytes and buffy coats – the portion of an anticoagulated blood sample that contains the majority of leukocytes and platelets following centrifugation - using a highly sensitive time-resolved Förster resonance energy transfer (TR-FRET) assay[15,16]. With a specialised form of immunofluorescence, the assay relies on a specific antibody pair: 2B7, which binds to N-terminal huntingtin; and MW1, which binds to expanded polyglutamine tracts. Using a small sample – five patients and four healthy controls – it was possible to clearly distinguish between diseased and healthy plasma, with no overlap between the measurements of each of the groups. This technique was then more extensively validated in isolated monocytes, B cells and T cells in eight pre-manifest gene expansion carriers, 18 manifest gene expansion carriers and 12 healthy controls[17]. Indeed, this latter study revealed significant differences not only between individuals with and without the HD mutation, but also across disease stages. Most interestingly, it showed differences between pre-manifest and manifest gene expansion carriers. Furthermore, the disease burden score – a measure of lifetime exposure to mHTT –and several neuroimaging variables of brain atrophy, such as caudate atrophy and ventricular expansion, were significantly correlated with mHTT measurements.

Using a homogeneous time-resolved Förster resonance energy transfer (HTRF), Moscovitch-Lopatin and colleagues verified the previous results first in a small cohort of buffy coat samples and then in peripheral blood mononuclear cells in a large multisite study, involving 228 healthy controls and 114 gene expansion carriers[18,19]. Despite its statistical power, this study was not able to correlate mHTT with disease burden score or with Unified Huntington's Disease Rating Scale (UHDRS) Total Motor Score (TMS), possibly because the majority of participants were pre-manifest gene expansion carriers, and only a minority had manifest HD. Still, it was possible to significantly differentiate groups of gene expansion carriers based on their probability of motor onset within two years and the amount of mHTT in their peripheral blood mononuclear cells, the first sign that this molecule may have some predictive value of the disease onset.

Subsequent studies of total huntingtin protein in peripheral blood showed no difference between gene expansion carriers and healthy controls[17,18], with the exception of one, which reported a decrease in total HTT using an enzyme-linked immunosorbent assay (ELISA)[20].

In 2015, Wild and colleagues developed a novel femtomolar-sensitive immunoassay based on the same antibody pair using a 'single molecule counting' (SMC) platform, and successfully quantified mHTT in cerebrospinal fluid for the first time in two different cohorts, from the United Kingdom and Canada[21]. With this new technique, virtually no mHTT signal was detected in healthy controls,

while most of the gene expansion carriers had mHTT detected in their CSF, with manifest carriers having higher levels than pre-manifest (Fig. 2). This study also revealed that in premanifest subjects the mHTT level in the CSF was correlated with their probability of onset, and in manifest subjects it was correlated with some clinical measures of disease severity, such as the Unified Huntington's Disease Rating Scale (UHDRS) Total Motors Score (TMS), the symbol-digit modality test, Stroop colour naming test, Stroop word reading test and Stroop interference test. Finally, the same study showed an association between mHTT and two measures of neuronal damage, neurofilament light chain (NfL) and total tau (Fig. 3).

Shortly after, another method was developed by Southwell et al. to measure mHTT in the CSF[22]. In the same Canadian cohort studied by Wild and colleagues, they showed, using more widely-available micro-bead based immunoprecipitation and flow cytometry methods, that mHTT in gene expansion carriers increases with disease stage and correlates with clinical measures (Fig.4). No signal was detected in healthy controls. They also demonstrated reduction in brain lysates and CSF mHTT after CNS administration of an HTT-lowering antisense oligonucleotide.

In the future we expect to see these results replicated in longitudinal studies examining the clinical predictive power of CSF mHTT. At least one mHTT assay is likely to be validated to regulatory standards for clinical trial use as a pharmacodynamic marker of HTT lowering.

3.2. Protein markers of neuronal damage

Neurodegeneration is readily detectable as an early feature of HD both pathologically[23] and non-invasively through neuroimaging[24]. Two major international collaborative studies, Track-HD [25-27,8] and Predict-HD[28], showed that disease-related atrophy of the striatum and white matter is present and detectable several years prior to the expected symptom onset, and that over the course of the disease, including in pre-manifest individuals, it is possible to measure neurodegeneration indirectly via yearly assessments of striatal, cortical and white-matter volumes. Biofluid biomarkers that accurately measure neuronal damage could still provide a cheaper, more rapid and more specific measure of disease progression or therapeutic efficacy. Largely informed by developments in other neurodegenerative diseases, some progress has been made in identifying biofluid biomarkers of neuronal damage in HD (Table 2).

The first such molecule to be tested was S100B in serum by Stoy and colleagues in 2005, using commercially-available ELISA assays[29]. S100B is a known marker of acute brain injury, released by astrocytes in response to the insult[30]. Unfortunately, no difference was detected between patients

with HD and healthy controls, probably indicating that this marker of acute brain damage is incapable of detecting the slow kinetics of neurodegeneration in HD.

Another important group of molecules associated with axonal injury and neurodegeneration are the neurofilament proteins of the axonal cytoskeleton[31]. Three isoforms exist: the heavy, the medium, and the light chain, and some have been associated with neurodegeneration in other conditions [32].

In 2007, Wild and colleagues measured neurofilament heavy chain in the plasma of 117 subjects, using an in-house ELISA assay[33]. No difference was found between gene expansion carriers and healthy controls, or between pre-manifest and manifest gene expansion carriers. In addition, no measure of the clinical phenotype was correlated with this isoform of neurofilament.

The light chain isoform was first detected and found to be significantly elevated in the CSF of HD patients in 2009 by Constantinescu and colleagues [34], using an in-house ELISA assay (Fig. 5). This group also established a positive correlation between the UHDRS Total Functional Capacity (TFC) and neurofilament light chain (NfL), but not with other clinical measures. Interestingly, Rodrigues and colleagues, making use of similar samples sizes and of a commercially available ELISA assay, showed that the neurofilament light chain was significantly different between healthy controls and gene expansion carriers, as it was between pre-manifest and manifest individuals, to a magnitude of difference having no overlap between the confidence intervals of these populations; and that age-adjusted neurofilament light chain is correlated with disease stage, measures of clinical motor phenotype severity and of functional ability[35]. In 2015 Wild and colleagues demonstrated that neurofilament light chain level was closely associated with that of mHTT in CSF[21].

Tau, an axonal protein with microtubule-stabilizing functions, is also considered a non-specific marker of neuronal damage[36]. The first report of the alteration of total tau concentrations in CSF was made by Constantinescu and colleagues who, by means of a commercially available ELISA assay, showed total tau was significantly elevated in patients compared with controls[37]. No correlations were found with clinical measures. A second report by Rodrigues and colleagues using two different cohorts, one from the United Kingdom and another from Canada, and another commercially available ELISA assay, reinforced the differences between gene expansion carriers and healthy controls, even after adjustment for age, and identified correlations between motor, cognitive and functional ability measures and total tau concentration in manifest gene expansion carriers, after age and disease burden adjustment (Fig. 6)[38]. A head-to-head comparison of NfL and Tau in CSF suggests that NfL is more strongly associated with clinical phenotype in HD[39]. Tau has been

directly implicated in the pathobiology of HD[40,41], so studying it in CSF may nonetheless come to have some role in dissecting the relative contributions of different pathways.

In 2015, single molecule assay ('SIMOA') technology facilitated successful quantification of NfL in blood for the first time[42]. Blood NfL levels have been shown to predict disease progression in several other neurodegenerative diseases including frontotemporal dementia, alzheimer's disease, amyotrophic lateral sclerosis and progressive supranuclear palsy[43-48].

In 2017 Byrne and colleagues quantified NfL in blood plasma in HD for the first time, in the large 366-participant TRACK-HD cohort[49]. Strikingly, plasma levels of NfL were significantly increased at every disease stage compared with controls, even in the early premanifest group, with significant differences even between early and late premanifest, and between late premanifest and early manifest HD (Fig.7). Plasma NfL was very closely associated with CAG repeat length, the first biofluid marker to show a genetic dose-response relationship with the causative expansion. Higher CAG repeat counts were associated with earlier and steeper increases in plasma NfL. (Fig.7). Baseline plasma NfL predicted subsequent disease onset within three years in premanifest mutation carriers, the first time a biofluid marker has shown such predictive value. Baseline NfL also predicted subsequent change in measures of cognitive and functional ability and brain atrophy (Fig.7), even after adjustment for the known predictors of HD – age and CAG repeat length. In a separate cohort, plasma and CSF levels of NfL were strongly correlated (Fig.7). While it requires further study especially in terms of the relative value of plasma and CSF, and the response to a successful treatment remains to be seen, NfL appears to be a robust biomarker of HD disease progression and neuronal damage.

3.3. Inflammatory markers

Immune system dysfunction has been implicated in neurodegeneration in several different conditions, including in the pathogenesis of primarily degenerative diseases[50]. HD is a special case, since the immune system appears to undergo primary derangement due to the presence of mHTT in leukocytes and microglia. This derangement, first uncovered by a proteomic biomarker study in plasma[51], gave rise to an independent field of study that has ultimately led to at least one drug trial of an immunomodulatory agent[52-54]. The immune system has some potential as a source of biomarkers of natural history in HD (Table 3), as well as pharmacodynamic markers of immune-targeting therapeutics, but its potential is limited by the propensity of such markers to be altered by infections and other derangements unrelated to HD pathology.

Importantly, a key unanswered question regarding this class of biomarkers is whether these changes represent primary peripheral abnormalities due to ubiquitous expression of mHTT, transfer of inflammatory molecules to the periphery across the blood–brain barrier, or a combination of both. These limitations restrict the utility of such biomarkers in the clinical trial setting.

Hyperactivity of myeloid cells including microglia, and subtle alterations of the innate immune system, are among the first biological changes so far detected in people with the HD mutation. Björkqvist, Wild and colleagues described in 2008 that IL-6 is increased in plasma of pre-manifest and manifest gene expansion carriers (Fig. 8) and this alteration can be found, on average, 16 years before the predicted onset of clinical symptoms[55]. This result was attained using 194 plasma samples and a Mesoscale Discovery electrochemoluminescence assay. Interestingly, in this study CSF concentrations were correlated with plasma concentrations. Still, this analyte is known to cross the blood-brain barrier and its concentration may reflect systemic alterations or parallel CNS and peripheral inflammation[56,55]-

CSF YKL-40 (also known as Chitinase-3-like protein 1, CHI3L1) – a poorly understood inactive enzyme associated with astrocytes and microglia [57,58] – has shown mixed results in HD [59,60], but encouragingly, a pilot study by our group has demonstrated a strong association with disease stage and motor and functional severity/phenotype[59]. Furthermore, we showed that these correlations were independent from disease burden score (Fig. 9), a property only attained by the mHTT itself, and other markers of neuronal damage.

Clusterin, also known as Apolipoprotein J, is a chaperone glycoprotein that has shown promising results as a general biomarker of neurodegeneration and a genetic modifier of Alzheimer's disease, through unclear mechanisms [ref]. In HD, a moderately sized study explored plasma and CSF of people with HD and healthy controls using ELISA techniques. In two different cohorts, one from England and another from Canada, this molecule was found to be increased in HD[51]. A second 79-participant study using ELISA in serum rather than plasma did not replicate these results[61]. Still, further work is needed to understand the validity of this biomarker.

3.4. Transglutaminase activity

Transglutaminases are enzymes which facilitate linkage reactions between glutaminyl- and lysyl-containing molecules or polyamines. *In vitro* experiments have shown that expanded polyglutamine – which confers toxicity upon mHTT protein – strengthens transglutaminase activity, suggesting transglutaminases may be involved in the regulation of mHTT aggregation. Further supporting this

hypothesis, the treatment of HD transgenic mice with cystamine –a known inhibitor of transglutaminase – alleviated HD symptoms[62].

Jeitner and colleagues have investigated several suggested markers for transglutaminase activity, including Nε-(γ-l-Glutamyl)-l-lysine (GGEL)[63,64], γ-Glutamylspermidine, γ-Glutamylputrescine and bis-γ-Glutamylputrescine[64], in the CSF of HD patients (Table 4). All markers were higher in HD patients compared with controls (hospitalized for unspecified spinal injury) after quantification using high-performance liquid chromatography (HPLC). Despite this seemingly supporting evidence, transglutaminase activity has thus far disappointed as a clinically relevant therapeutic target. A randomized, placebo-controlled, phase 2/3 clinical trial of cysteamine (CYST-HD) in HD patients did not meet its primary endpoint[65].

3.5. Neurotransmitters

Some of earliest studies examining biofluid biomarkers in HD took a particular interest in neurotransmitters, neuromodulators and neuropeptides as surrogate outcome measures of neuronal viability and function (Table 5).

One of the pathological hallmarks of HD is loss of GABAergic medium spiny neurons[66], triggering studies of the GABAergic system. Enna and colleagues were the first to successfully detect GABA in CSF from HD patients, in 1977, using a radioreceptor assay[67]. Lower concentrations of this neurotransmitter were detected in several biomarker studies evaluating CSF and blood of people with HD when comparing with controls[67-71]. It is noteworthy that these results are inconsistent across all published evidence[72,73]. The investigation of homocarnosine, a dipeptide containing GABA, also showed contradictory results in the CSF. Some studies pointed towards a significant increase of this molecule while others towards a significant decrease[70,72]. Further disappointment was attained after results from a randomized, double-blinded, placebo-controlled trial in patients using isoniazid and γ-acetylenic GABA, showing that CSF but not plasma GABA levels increased in response to this antibiotic[74], and CSF concentrations of GABA and homocarnosine levels increased but with no significant clinical improvement[75].

The basal ganglia have a high concentration of cholinergic neurons and cholinesterase activity, and the most affected nuclei in HD are the caudate and the putamen. Comparisons between choline levels in patients with HD and healthy controls generated inconsistent results in CSF[76,77], and cholinesterase activity was not different between these two groups[76,78,77]. Results from the

isoniazid trial above showed that this molecule did not affect CSF choline level or cholinesterase activity[77].

The striatum receives dopaminergic input from the substantia nigra. Neuronal loss in the striatum – and therefore loss of postsynaptic striatal dopamine receptors – during the course of HD creates an imbalance due to a reduction in dopamine uptake. This disproportion was thought to contribute to chorea on the basis of an overactive effect of dopamine on the striatum[79]. Dopamine metabolites were therefore a source of early interest as possible biofluid biomarkers.

However, CSF levels of dopamine and its metabolites showed challenging results[80,81]. Homovanillic acid (HVA) – a result of dopamine metabolism and maker of dopaminergic activity, has been extensively studied both in the CSF, blood and urine of patients with HD. Again, the results were disappointing, with some studies showing a decrease[81-83], while others showed no difference[84,80,85,86], and only one study supported the theory of dopaminergic overactivation[87]. Several other dopamine metabolites were also studied extensively with no definitive contribution towards the dopaminergic activity hypothesis[88,82,89,90,81,85,91].

3.6. Transcriptomic and proteomic approaches

Several exploratory transcriptomic (Table 6) and proteomic and (Table 7) approaches to HD pathobiology and biomarkers have been investigated. These enable the hypothesis-free exploration of biochemical differences between HD and control biofluids but in isolation present difficulties of extracting signal from noise and require replication in hypothesis-driven mechanistic studies, which have been lacking. Unfortunately, little from these approaches has been translated into the pursuit of new therapeutic pathways.

HD is known for transcriptional dysfunction which may be due to interactions between mHTT and DNA. Using microarray technology, one group identified transcriptional differences in blood in a panel of 12 genes, between controls and gene expansion carriers[92]. Unfortunately others could not externally validate these results[93]. This finding may be explained by genetic, environmental, medication or dietary differences between populations, or the inherent statistical and technical challenges of transcriptomics. The apparent failure of gene expression profiling to produce readily measured biomarkers is disappointing, but as larger populations are studied using more recent methods, useful transcriptomic biomarkers may yet emerge.

Proteomic studies in CSF, blood and urine have largely suffered from the same conceptual concerns and lack of validation. One exception is immune dysfunction (see above), which was first identified using plasma proteomics[51].

3.7. Kynurenine pathway metabolites

Before the identification of *HTT* as the cause of HD, quinolinic acid (QA) lesioned mice were a common experimental model used to study HD as they displayed a similar pathology to HD patients. QA is a key component of the kynurenine pathway (KP), a biochemical pathway encompassing the oxidative metabolism of tryptophan. QA's agonistic action on NMDA receptors gives it a selective toxicity for medium spiny neurons produced after direct intra-striatal injection. This discovery inspired much interest in QA and other KP metabolites (Table 8) and introduced the concept of excitotoxicity as a disease mechanism in HD.

Some of the earliest studies of KP metabolites in humans examined CSF, driven by the effects of QA injection in mouse striatum. QA, kynurenic acid (KA) and tryptophan have all been reported to be altered in CSF from HD patients[94-97]. Unfortunately, all studies of KP metabolites in CSF thus far were carried out before the discovery of the causative gene for HD. They also used inconsistent methods and sample collection procedures such as collecting at different times of day and with or without fasting.

Interest in this pathway has been revisited more recently in blood. In 2005, Stoy and colleagues used HPLC to quantify the levels of six major components of the kynurenine pathway – tryptophan, kynurenine, KA, 3-hydroxykynurenine, 3-hydroxyanthranilic acid (3HAA) and xanthurenic acid – in the blood of eleven hospitalised advanced HD patients and 15 healthy controls after loading and depletion of tryptophan[29]. The kynurenine:tryptophan and KA:kynurenine ratios were increased, while levels of the redox-active 3HAA was decreased in HD patients compared to controls suggesting a greater conversion of tryptophan to kynurenine in patients. These changes in KP metabolites supported previously published measurements of their levels from post-mortem brain or animal models[94,97-101]. Forrest, Mackay and colleagues (2010) found levels of tryptophan were lower and the kynurenine:tryptophan ratio was higher in HD patients than in healthy controls, supporting results previously seen by Stoy et al. (2005)[102,29].

An important caveat when interpreting evidence from KP metabolites in blood is that they do not have equal ability to cross the blood-brain barrier. For example tryptophan and kynurenine readily pass through neutral amino acid transportors[103-105], in contrast, other components pass poorly

by diffusion, such as 3HAA and KA. This raises the question of whether blood is a relevant medium to measure disease markers of HD-related neuropathology. Blood also may be too dynamic for measuring metabolic markers to give a meaningful indication about HD disease processes.

There remains strong evidence for the KP's involvement in HD but the studies currently available to us are insufficient to provide a conclusive answer to how KP metabolites may be involved in HD pathology. It will be important to further investigate KP metabolites in CSF using high-quality CSF with matched blood samples from a large and clinically well-characterised cohort with contemporaneous healthy control samples for comparison.

3.8. Oxidative stress

Oxidative stress has been associated with several neurodegenerative conditions[106-108]. HD is no exception, and CSF, blood and urine have been used to investigate markers of oxidative stress in this population[109,110]. Two relatively well-characterized substances are F2-isoprostanes and 8-Hydroxy-2-deoxyguanosine (8OH2'dG), but multiple others have been investigated (Table 9).

F2-isoprostanes are a marker for lipid peroxidation. In a small population of patients with HD, and healthy and disease controls, Montine and colleagues found that HD CSF contained an excess of these markers[109], a result in line with previous research in Alzheimer's disease[106]. Further investigation by the same group with blood and urine samples, also using gas chromatography/negative ion chemical ionization mass spectrometry could not replicate such abnormalities[110].

Based on the findings from animal models and human observational studies, a number of randomized clinical trials invested in compounds hypothesised to act as anti-oxidants, such as ethyl-EPA[111-113], creatine[114,115] and coenzyme Q10[116-118]. The results were disappointing in terms of clinical efficacy but data on several potential biomarkers was obtained. In particular, Hersch and colleagues found great differences between patients and controls in 8OH2'dG levels[114], a change that has also been found incidentally by others[119]. This molecule is proposed to be an indirect measurement of oxidative damage to DNA. Indeed, creatinine was theorized to be able to change the levels of 8OH2'dG in HD, and consequently the intensity of DNA damage. Further studies did not validate these findings[120,121,115], and it is now well-established from rigorously-conducted large-scale replication studies that 8OH2'dG is not a disease biomarker in HD (Fig. 10)[122].

3.9. Neuroendocrine and metabolic markers

Both wild type and mutant huntingtin are expressed throughout most body tissues[123], perhaps accounting for at least some of the neuroendocrine and metabolic abnormalities found in HD such as weight loss and muscle atrophy[124]. On one hand, neuronal populations responsible for coordinating and giving feedback to complex endocrine loops may be subject to dysfunction and neurodegeneration; on the other, tissues outside the CNS also show susceptibility to mHTT accumulation and eventually dysfunction[125].

For these reasons, neuroendocrine and metabolic biofluid biomarkers are not only seen as potential surrogates for clinical outcome measures in research and clinical practice, but as useful hints in the preclinical development of therapeutic approaches to some of the peripheral abnormalities found in people with HD (Table 10 and Table 11). So far more than fifty such molecules have been examined in humans with HD. For a comprehensive review on this topic please refer to references[126,124].

BDNF, or brain derived neurotrophic factor, is a peptide of utmost relevance to the survival of striatal neurons. It was found decreased in the blood of gene-positive individuals (Fig. 11)[127,128]. Indeed some authors showed these alterations were correlated with the clinical phenotype[127], and were already present in premanifest individuals[129]. However, these findings were not corroborated in a larger study involving 398 indivuduals[130], mainly because, although blood concentration reflects at least partially in-brain concentration[131], this factor is also produced peripherally by megakaryocytes and platelets, and variations in blood collection, storage and measurement techniques have a significant impact in the final results[130]. In 2014, another moderately sized study refuted the conclusion of the first reports[132]. No study has so far explored BDNF in CSF.

Melatonin is a light-sensitive hormone mainly secreted by the pineal gland. It has an important role in the sleep-wake cycle, which is highly deregulated early in the course of HD[133]. Two preliminary reports showed no differences in plasma melatonin in people with HD[91,134]. A third study in a larger cohort showed that the mean 24-hour hormone concentration decreases with disease progression, as does the amount secreted during the acrophase of its production – during the night[135]. This was seen even in premanifest individuals.

These endocrine abnormalities are of clinical interest and may yield useful biomarkers. Some endocrine abnormalities may be amenable to treatment using existing therapies, which may have both central and peripheral benefits. If treatable, measures of endocrine dysfunction will become biomarkers of pharmacodynamics rather than of progression. Endocrine features are highly

susceptible to influences other than pathology, such as drugs and depression; this must be borne in mind when assessing their utility as potential biomarkers. As with so many other proposed markers, proper validation studies of the assays and the disease-related changes are lacking.

4. Conclusion

Many biofluid biomarker candidates have been reported over several decades of HD research. The most noteworthy and most likely to be genuinely useful in the near future include:

- Mutant huntingtin and other huntingtin species in CSF, with the immediate demand for pharmacodynamics and target-engagement markers for current and upcoming clinical trials of huntingtin-lowering therapeutics;
- Neuronal damage markers in CSF and blood – especially neurofilament light protein – which have demonstrated neuropathological relevance and the accumulating evidence of prognostic value from studies in HD and other neurodegenerative diseases.

Many methodologies have been explored from radioenzymatic assays to enhanced immunoassays producing ultrasensitive quantification. As technology continues to improve, the potential for more sensitive and specific techniques which could unearth biofluid markers of previously immeasurable alterations increases. A real-life example of this in HD is the quantification of mHTT itself. Single molecule counting (SMC) technology made it possible for the first time to measure mHTT in CSF, even in premanifest individuals with extremely low concentrations[21]. Most recently SIMOA technology which has uncovered a robust blood-based biomarker of neuronal damage in neurofilament light chain[49].

Methodologies and disease-related findings both require validation, particularly if they are to be considered for use as clinical trial endpoints. This has not yet been achieved for any of the biofluid biomarkers that we have discussed in this chapter. The closest to validation in HD are assays for huntingtin and the protein markers of neuronal damage such as neurofilament light chain, which are already useful as exploratory endpoints. In order to achieve this level of validation, three things are necessary:

(1) A rigorous framework. The European Medicines Agency (EMA) and Food and Drug administration (FDA) provide guidelines on how to technically validate an assay, ensuring it will be robust and methodologically sound[136,137].

(2) Large observational studies designed for biomarker evaluation. Disease-related findings from smaller studies need to be replicated in cohorts with large numbers of samples and ideally from multi-site studies with complete consistency between sites and comprehensive clinical data. Samples collected in large-scale observational studies in HD such as TRACK-HD and Predict-HD offer a current source to do this for potential blood biomarkers. Previously, studies of potential CSF biomarkers for HD have been thwarted by low sample numbers and inconsistent collection procedures. HDClarity, a multi-site CSF collection initiative for HD, will be collecting CSF and blood samples from 600 subjects across all disease stages as well as healthy controls. This will provide an invaluable resource to facilitate the validation of both blood and CSF biomarkers for HD[138].

(3) Motivation. Biomarkers do not discover themselves, and nor do findings replicate themselves. For any biofluid biomarker of HD to make it as a useful tool clinically or commercially, there has to be rigorous work to move from hypothesis-driven discovery work to the replication of methods and findings to regulatory standards in large-scale cohorts.

The last five years alone have seen significant progress with biofluid biomarkers having real potential to study and predict HD disease progression and therapeutic response. With concerted work, it is to be hoped that the years to come will see some of these biofluid biomarkers reaching validation for use in the therapeutic trial or even clinical setting.

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Figures Captions

Fig. 1. Timeline of published CSF biomarkers in Huntington's disease. Triangular markers indicate the identification of the HTT gene as the cause of HD. Adapted from Byrne et al.[10] with the author's and publisher's permission.

Fig. 2. In two independent cohorts (A and B), CSF mHTT levels were different between gene expansion carriers and healthy controls, and premanifest and manifest gene expansion carriers. Adapted from Wild et al.[21] with the author's and publisher's permission.

Fig. 3. CSF mHTT levels correlate with CSF markers of neuronal death, tau (A, B) and NFL (C, D), in independent cohorts. Adapted from Wild et al.[21] with the author's and publisher's permission.

Fig. 4. Using a different assay than the one used by Wild et al., Southwell et al. showed that CSF mHTT levels change with disease progression (A), and correlates with several clinical measures, such as the verbal fluency (B), the symbol digit modality test (C), the Stroop colour naming test (D), and the total motor score (E). Adapted from Southwell et al.[22] with the publisher's permission.

Fig. 5. CSF Neurofilament light chain (NfL) is increased in patients with Huntington's disease. Adapted from Constantinescu et al.[34] with the publisher's permission.

Fig. 6. (a) CSF tau levels are higher in gene expansion carriers comparing with healthy controls, and correlates with clinical measures, such as the total functional capacity (b), and the total motor score (c). Adapted from Rodrigues et al.[38] with the author's and publisher's permission.

Fig. 7. (A) Plasma neurofilament light chain (NfL) is increased in Huntington's disease and rises with advancing disease. (B) Each CAG increase is associated with higher, more steeply rising NfL. (C) Baseline plasma NfL is able to predict subsequent cognitive, motor and functional decline, and brain atrophy. (D) Baseline plasma NfL predicted subsequent onset of manifest HD in the mutation carriers who were premanifest at the start of the study, even after adjustment for age, CAG and baseline brain volumes. (E) CSF NfL is closely correlated with plasma NfL. Adapted from Byrne et al.[49] under a creative commons licence.

Fig. 8. The plasma concentration of IL-6 is higher is gene expansion carriers. Adapted from Björkqvist et al.[55] with the publisher's permission.

Fig. 9. YKL-40 shows a strong correlation with phenotypic measurements. Adapted from Rodrigues et al.[38] with the author's and publisher's permission.

Fig. 10. 8OHdG measure with (A) liquid chromatography–electrochemical array (LCMS) and with (B) liquid chromatography–mass spectrometry (LCECA) does not change with disease progression. Adapted from Borowsky et al.[122] with the publisher's permission.

Fig. 11. Brain-derived neurotrophic factor is decreased in patients with Huntington's disease. Adapted from Ciammola et al.[127] with the publisher's permission.

Tables

Table 1. Summary of published studies reporting HTT as a biofluid biomarker in HD.

Molecule	Sample	Study	n	Direction	Method
HTT protein	Blood	Weiss 2012 [17]	40	C = HD	TR-FRET
		Moscovitch-Lopatin 2010 [19]	38	C < HD	HTRF
		Moscovitch-Lopatin 2013 [18]	342	C = HD	HTRF
		Massai 2013 [20]	25	C > HD	ELISA
mHTT protein	CSF	Southwell 2015 [22]	37	C < HD PM = M	IP-FCM
		Wild 2015 [21]	52	C < HD PM < M Corr stage Corr NFL and tau	Singulex SMC Immunoassay
	Blood	Weiss 2009 [15]	9	C < HD	TR-FRET
		Moscovitch-Lopatin 2010 [19]	38	C < HD	HTRF
		Weiss 2012 [17]	40	C < HD PM < M	TR-FRET
		Moscovitch-Lopatin 2013 [18]	342	C < HD	HTRF
		Wild 2015 [21]	52	CSF corr plasma	Singulex SMC Immunoassay

Abbreviations: n, sample size; HTT, Huntingtin; mHTT, mutant Huntingtin; C, healthy controls; HD, Huntington's disease gene expansion carriers; PM, premanifest gene expansion carriers; M, manifest gene expansion carriers; CSF, cerebrospinal fluid; Corr, correlates; TR-FRET, Time resolved fluorescence resonance energy transfer; HTRF, homogeneous time-resolved fluorescence; ELISA, enzyme-linked immunosorbent assay; IP-FCM, micro-bead based immunoprecipitation and flow cytometry; NFL, neurofilament light chain; SMC, single-molecule counting mHTT immunoassay.

Table 2. Summary of published studies on protein markers of neuronal death in HD.

Molecule	Sample	Study	n	Direction	Method
NfL	CSF	Constantinescu 2009 [34]	35	C < HD Corr TFC	ELISA
		Rodrigues 2016[35]	37	C < HD PM < M Corr stage Corr TFC Corr TMS	ELISA
		Niemela 2017[39]	23	PM < M Corr TFC Corr TMS Corr tau	ELISA
		Byrne 2017[49]	37	Corr plasma NfL	SIMOA
	Blood	Byrne 2017[49]	298	C < HD Corr stage Corr TMS Corr cogn Corr imaging	SIMOA
NfH	Blood	Wild 2007 [33]	117	C = HD	ELISA
Tau	CSF	Constantinescu 2011 [37]	35	C < HD	ELISA
		Rodrigues 2016 [38]	67	C < HD Corr TFC Corr TMS Corr Cogn	ELISA
		Niemela 2017 [39]	23	Corr TFC Corr TMS Corr NfL	ELISA
S100B	Blood	Stoy 2005 [29]	26	C = HD	ELISA
		Silajdzic 2013 [61]	Cohort1 79 Cohort2 42	C = HD C = HD	Luminex

Abbreviations: n, sample size; HTT, Huntingtin; mHTT, mutant Huntingtin; C, healthy controls; HD, Huntington's disease gene expansion carriers; PM, premanifest gene expansion carriers; M, manifest gene expansion carriers; CSF, cerebrospinal fluid; Corr, correlates; TFC, UHDRS Total Functional Capacity; TMS, UHDRS Total Motor Score; Cogn, measures of cognition; ELISA, enzyme-linked immunosorbent assay.

Table 3. Summary of published studies on inflammatory biofluid biomarkers in HD.

Molecule	Sample	Study	n	Direction	Method
IL-1	CSF	Rodrigues 2016 [59]	37	Not detected	MSD antibody-based tetraplex array
	Blood	Mochel 2007 [139]	53	C = HD	ELISA
		Bjorkqvist 2008 [55]	194	C = HD	MSD immunoassay
		Wang 2014 [132]	39	C = HD	Human cytokine/chemokine assay kit
IL-2	Blood	Bjorkqvist 2008 [55]	194	C = HD	MSD immunoassay
IL-2 receptor	Blood	Leblhuber 1998 [140]	23	C < HD	ELISA
IL-4	Blood	Bjorkqvist 2008 [55]	194	C = HD Corr stage	MSD immunoassay
IL-5	Blood	Bjorkqvist 2008 [55]	194	C = HD	MSD immunoassay
IL-6	CSF	Bjorkqvist 2008 [55]	194	Corr plasma IL-6	ELISA
		Rodrigues 2016 [59]	37	C < HD	MSD antibody-based tetra-plex array
	Blood	Dalrymple 2007 [51]	96	C < HD	ELISA
		Mochel 2007 [139]	53	C = HD	ELISA
		Bjorkqvist 2008 [55]	194	C < HD PM < M Corr stage	MSD immunoassay
		Sanchez-Lopez 2012 [141]	23	C < HD	ELISA
		Trager 2014 [142]	80	C < HD	MSD immunoassay
		Wang 2014 [132]	39	C = HD	Human cytokine/chemokine assay kit
		Chang 2015 [143]	36	C < HD Corr IS Corr TFC PM = M	ELISA
IL8	CSF	Bjorkqvist 2008 [55]	194	Corr plasma IL-6	ELISA
		Rodrigues 2016 [59]	37	C =HD	MSD antibody-based tetra-plex array
	Blood	Bjorqvist 2008 [55]	194	C < HD Corr stage Corr TFC Corr TMS	MSD immunoassay
IL-10	Blood	Bjorqvist 2008 [55]	194	C = HD Corr stage	MSD immunoassay
		Wang 2014 [132]	39	C = HD	Human cytokine/chemokine assay kit
IL-12	Blood	Bjorqvist 2008 [55]	194	C = HD	MSD immunoassay
IL-13	Blood	Bjorqvist 2008 [55]	194	C = HD	MSD immunoassay
IL-16	Blood	Chang 2015 [143]	36	C = HD PM = M	ELISA
IL-18	Blood	Chang 2015 [143]	36	C > HD PM = M	ELISA
IL-23	Blood	Forrest 2010 [102]	113	C < HD Corr stage Corr CAG	Immunoassay kit
sCD23	Blood	Leblhuber 1998 [140]	23	C = HD	ELISA
sHLA-G	Blood	Forrest 2010 [102]	113	C = HD	ExBIO/BioVendor ELISA assay kit

TNF-α	CSF	Rodrigues 2016 [59]	37	Not detected	MSD antibody-based tetra-plex array
	Blood	Bjorqvist 2008 [55]	194	C = HD Corr stage Corr TFC Corr TMS	MSD immunoassay
		Wang 2014 [132]	39	C = HD	Human cytokine/chemokine assay kit
sTNF receptor	Blood	Leblhuber 1998 [140]	23	C < HD	ELISA
INF-γ	Blood	Bjorqvist 2008 [55]	194	C = HD	MSD immunoassay
GM-CSF	Blood	Bjorqvist 2008 [55]	194	C = HD	MSD immunoassay
MMP-3/-9/-10	Blood	Chang 2015 [143]	36	MMP-3: C < HD PM = M MMP-9: C = HD PM = M MMP-10: C = HD PM = M	ELISA
TIMP-2	Blood	Chang 2015 [143]	36	C = HD PM = M	ELISA
VEGF	Blood	Chang 2015 [143]	36	C < HD PM = M	ELISA
TGF-1β	Blood	Squitieri 2009 [144]	N/S	C = HD	N/S
		Battaglia 2011 [145]		C > HD PM < M	TGF-1β Emax Immunoassay System kit
		Chang 2015 [143]	36	C < HD PM = M	ELISA
MIP-1α/-3β	Blood	Chang 2015 [143]	36	C = HD PM = M	ELISA
Clusterin	CSF	Dalrymple 2007 [51]	29	C < HD	ELISA
	Blood	Dalrymple 2007 [51]	20	C < HD	2D electrophoresis
			90	C < HD	Semiquantitative immunoblotting
			73	C < HD	ELISA
		Silajdzic 2013 [61]	Cohort1 79	C = HD	ELISA
VCAM-1	Blood	Chang 2015 [143]	36	C = HD PM = M	ELISA
ICAM-1	Blood	Chang 2015 [143]	36	C = HD PM = M	ELISA
β-actin	Blood	Dalrymple 2007 [51]	20	C < HD	2D electrophoresis
YKL-40	CSF	Vinther-Jensen 2014 [60]	68	C = HD PM = M Corr TMS	ELISA
		Rodrigues 2016 [59]	37	C < HD Corr stage Corr TFC Corr TMS Corr NFL+Tau	MSD antibody-based tetra-plex array
	Blood	Vinther-Jensen 2014 [60]	68	C = HD	ELISA
Chitotriasidase	CSF	Rodrigues 2016 [59]	37	C < HD	Mattsso 2011 [146]
Prothrombin	CSF	Huang 2011 [147]	12	C < HD	Two-dimensional electrophoresis and mass spectrometry
		Huang 2011 [147]	18	C < HD Corr TMS	ELISA

				Corr IS	
	Blood	Huang 2011 [147]	18	C = HD	ELISA
Haptoglobin	CSF	Huang 2011 [147]	12	C < HD	Two-dimensional electrophoresis and mass spectrometry
		Huang 2011 [147]	18	C < HD	ELISA
	Blood	Huang 2011 [147]	18	C = HD	ELISA
Neopterin	Blood	Leblhuber 1998 [140]	23	C < HD Corr cogn	ELISA
		Stoy 2005 [29]	26	C < HD Corr CRP	ELISA
		Christofides 2006 [91]	26	C < HD	ELISA
Immunoglobulins A, M , G	Blood	Leblhuber 1998 [140]	23	IgA: C < HD IgM: C = HD, corr cogn IgG: C = HD	Nephelometry and indirect immuno-fluorescence
		Bjorqvist 2008 [55]	194	C = HD	Single radial immunodiff-usion assays
C3	Blood	Leblhuber 1998 [140]	23	C < HD	Nephelometry and indirect immuno-fluorescence
		Silajdzic 2013 [61]	Cohort1 79 Cohort2 42	C = HD C = HD	Luminex and MRM
C4	Blood	Leblhuber 1998 [140]	23	C = HD	Nephelometry and indirect immuno-fluorescence
		Silajdzic 2013 [61]	Cohort1 79 Cohort2 42	C = HD C = HD	Luminex
C5	Blood	Silajdzic 2013 [61]	Cohort1 79	C = HD	MRM
C7	Blood	Dalpymple 2007 [51]	109	C < HD	2D electrophoresis
C9	Blood	Dalpymple 2007 [51]	109	C < HD	2D electrophoresis
		Silajdzic 2013 [61]	Cohort1 79	C = HD	MRM
Complement factor B	Blood	Silajdzic 2013 [61]	Cohort1 79	C = HD	MRM
Complement factor H	Blood	Silajdzic 2013 [61]	Cohort1 79 Cohort2 42	C = HD C ≠ HD	Luminex and MRM
Eotaxin 3	Blood	Wild 2011 [148]	Cohort1 99 Cohort2 94	C < HD C < HD	ELISA
MIP-1β	Blood	Wild 2011 [148]	Cohort1 99 Cohort2 94	C < HD C < HD	ELISA
MIP-4 (CCL18)	Blood	Silajdzic 2013 [61]	Cohort1 79	C = HD	Luminex
Eotaxin	Blood	Wild 2011 [148]	Cohort1 99 Cohort2 94	C = HD C < HD	ELISA
MCP-1	Blood	Wild 2011 [148]	Cohort1 99 Cohort2 94	C = HD C = HD	ELISA
MCP-4	Blood	Wild 2011 [148]	Cohort1 99 Cohort2 94	C = HD C = HD	ELISA
Circulating immune complexes	Blood	Leblhuber 1998 [140]	23	C = HD Corr cogn	Nephelometry and indirect immuno-fluorescence
Cardiolipin	Blood	Leblhuber 1998 [140]	23	Corr cogn	Nephelometry and indirect immuno-fluorescence
CRP	Blood	Leblhuber 1998 [140]	23	C = HD	N/S

		Stoy 2005 [29]	26	C < HD Corr ESR	Behring Turbitimer
		Mochel 2007 [139]	53	C = HD	ELISA
		Sanchez-Lopez 2012 [141]	23	C < HD	Assay kit (OSR6147)
		Krzyszton-Russjan 2013 [149]	57	C = HD	N/S
		Silajdzic 2013 [61]	Cohort1 79 Cohort2 42	C > HD C = HD	Luminex and MRM
		Wang 2014 [132]	39	C < PM	ALPCO ELISA kit
		Bouwens 2014 [150]	164	C = HD	COBAS INTEGRA 800 analyzer
ESR	Blood	Leblhuber 1998 [140]	23	C = HD	N/S
		Stoy 2005 [29]	26	C = HD Corr CRP	Starrsed Automated ESR machine
		Mochel 2007 [139]	53	C = HD	ELISA
		Krzyszton-Russjan 2013 [149]	57	C = HD	N/S
sHLA-G	Blood	Forrest 2010 [102]	113	C = HD	ELISA
Myeloperoxidase (MPO)	Blood	Tasset 2012 [128]	38	C = HD	N/S
		Sanchez-Lopez 2012 [141]	13	HD1 > HD2	Oxis International
Prealbumin	Blood	Silajdzic 2013 [61]	Cohort1 79	C = HD	Luminex
Albumin	Blood	Phillipson 1977 [151]	18	C = HD	Bromocresol Green dye-binding method
		Leblhuber 1998 [140]	23	C = HD	N/S
		Bouwens 2014 [150]	117	C > HD	Modular P systems
LDH	Blood	Tasset 2012 [128]	38	C < HD	Kit from Linear Chemicals S. L.
Lactate	Blood	Duran 2010 [152]	91	C < M	L-lactic acid Enzymatic Bioanalysis (Roche) kit
		Josefsen 2010 [153]	19	C = HD	Passonneau and Lowry, 1993 [154]
		Ciammola 2011 [155]	50	C = HD	Colorimetric assay
α2-antiplasmin	Blood	Dalpymple 2007 [51]	109	C < HD	2D electrophoresis
α2-macroglobulin	Blood	Dalpymple 2007 [51]	109	C < HD	2D electrophoresis
	Blood	Silajdzic 2013 [61]	Co 1 79 Cohort2 42	C = HD C = HD	Luminex

Abbreviations: n, sample size; HTT, Huntingtin; mHTT, mutant Huntingtin; C, healthy controls; HD, Huntington's disease gene expansion carriers; PM, premanifest gene expansion carriers; M, manifest gene expansion carriers; CSF, cerebrospinal fluid; Corr, correlates; TFC, UHDRS Total Functional Capacity; TMS, UHDRS Total Motor Score; IS, UHDRS Independence Scale; Cogn, measures of cognition; MSD, Meso ScaleDiscovery; ELISA, enzyme-linked immunosorbent assay; NFL, neurofilament light chain; MRM, multiple reaction monitoring

Table 4. Summary of published studies on transglutaminase activity biofluid biomarkers in HD.

Molecule	Sample	Study	n	Direction	Method
Nε-(γ-l-Glutamyl)-l-lysine (GGEL)	CSF	Jeitner 2008 [63]	63	C < HD	MS
		Jeitner 2008 [64]	45	C < HD	HPLC
γ-Glutamylspermidine	CSF	Jeitner 2008 [64]	45	C < HD	HPLC
γ-Glutamylputrescine	CSF	Jeitner 2008 [64]	45	C < HD	HPLC
bis-γ-Glutamylputrescine	CSF	Jeitner 2008 [64]	45	C < HD	HPLC

Abbreviations: n, sample size; C, healthy controls; HD, Huntington's disease gene expansion carriers; PM, premanifest gene expansion carriers; M, manifest gene expansion carriers; CSF, cerebrospinal fluid; MS, mass spectrometry; HPLC, high-performance liquid chromatography.

Table 5. Summary of published studies on neurotransmitter studies in HD.

Molecule	Sample	Study	n	Direction	Method
GABA	CSF	Enna 1977 [67]	45	C > HD	Radioreceptor assay
		Manyam 1978 [68]	11	C > HD	Ion-exchange fluorometry
		Bohlen 1980 [69]	34	C > HD	HP cation exchange chromatography
		Uhlhaas 1986 [70]	58	C > HD	Ion-exchange chromatography
		Bonnet 1987 [72]	33	C < HD	HPLC
		Nicoli 1993 [73]	23	C = HD	High resolution proton NMR spectroscopy and HPLC
	Blood	Uhlhaas 1986 [71]	58	C > HD	Ion-exchange chromatography
		Nicoli 1993 [73]	23	C = HD	High resolution proton NMR spectroscopy and HPLC
Homocarnosine	CSF	Bohlen 1980 [70]	34	C > HD	HP cation exchange chromatography
		Bonnet 1987 [72]	33	C < HD	HPLC
Neuropeptide Y	CSF	Wagner 2016 [156]	30	C < HD	Competitive radioimmunoassay
Agouti-related protein	Blood	Wang 2014 [132]	39	C < PM C = M PM > M	Human Brain-Derived/ Pituitary Protein Multiplex Panel assay kit
Choline	CSF	Consolo 1977 [76]	27	C = HD	Radiochemical micromethod [157]
		Manyam 1990 [77]	15	C > HD	Radiometric enzymatic assay
AChE activity	CSF	Consolo 1977 [76]	27	C = HD	Radiochemical method [158]
		Manyam 1990 [77]	15	C = HD	Radiometric method [159]
	Blood	St Clair 1986 [78]	49	C = HD	Monoclonal Antibody Assay
Dopamine	CSF	Garret 1992	23	C < HD	HPLC

		[81]			
	Blood	Belendiuk 1980 [80]	76	C = HD	[160]
DBH	Plasma	Caraceni 1977 [89]	91	C = HD	[161]
Epinephrine	Blood	Belendiuk 1980 [80]	76	C < HD	[160]
	Urine	McNamee 1971 [88]	54	C = HD	[162]
Norepinephrine	CSF	Garret 1992 [81]	23	C = HD	HPLC
	Blood	Belendiuk 1980 [80]	76	C = HD	[160]
	Urine	McNamee 1971 [88]	54	C = HD	[162]
Tryptophan	See oxidativ e stress				
Serotonin	Blood	Belendiuk 1980 [80]	76	C < HD	Fluorometry
		Christofides 2006 [91]	26	C = HD	HPLC
HVA	CSF	Klawans 1971 [86]	13	C = HD	Fluorimetric method [163]
		Curzon 1972 [82]	26	C > HD Corr severity	[164]
		Garret 1992 [81]	23	C > HD	HPressureLC
		García Ruiz 1995 [85]	35	C = HD	HPressureLC
		Caraceni 1977 [83]	17	C > HD	[165]
	Blood	Belendiuk 1980 [80]	76	C = HD	HPLC
		Markianos 2009 [87]	169 ?	C=PM C < M Corr stage	HPLC

	Urine	Williams 1961 [84]	N/S	C = HD	N/S
VMA	Urine	McNamee 1971 [88]	54	C = HD	[166]
5-HIAA	CSF	Curzon 1972 [82]	39	C < HD	[167]
		Caraceni 1977 [89]	17	C > HD	[165]
		Kurlan 1988 [90]	77	C = HD	HPLC
		Garret 1992 [81]	23	C = HD	HPLC
		García Ruiz 1995 [85]	35	C = HD	HPressureLC
	Blood	Christofides 2006 [91]	26	C = HD	HPLC
	Urine	McNamee 1971 [88]	54	C = HD	[168]
DOPAC	CSF	Garret 1992 [81]	23	C < HD	HPLC
MHPG	CSF	Garret 1992 [81]	23	C < HD	HPLC
Monoamine oxidase	Blood	Belendiuk 1980 [80]	76	C < HD	[169]
A2A receptors	Blood	Varani 2003 [170]	115	C < HD	Multiple methods
		Maglione 2005 [171]	74	Corr anticipati on	Binding Assay
		Maglione 2006 [172]	94	C < HD Corr CAG Corr age onset	Radioligand binding assays
		Varani 2007 [173]	252	C < HD	Multiple methods
Histamine	Urine	McNamee 1971 [88]	54	C > HD	[174]
1,4-methylimidazoleac etic acid	Urine	McNamee 1971 [88]	54	C > HD	[174]

1,5-methylimidazoleacetic acid	Urine	McNamee 1971 [88]	54	C > HD	[174]
Kainic acid-like molecules	CSF	Beutler 1981 [175]	5	C = HD	Competition assay
	Blood	Beutler 1981 [175]	5	C = HD	Competition assay
	Urine	Beutler 1981 [175]	5	C = HD	Competition assay
Glycine	CSF	Nicoli 1993 [73]	23	C < HD	High resolution proton NMR spectroscopy and HPLC
	Blood	Reilmann 1997 [176]	37	C = HD	High-pressure liquid chromatography
		Nicoli 1993 [73]	23	C = HD	High resolution proton NMR spectroscopy and HPLC

Abbreviations: n, sample size; C, healthy controls; HD, Huntington's disease gene expansion carriers; PM, premanifest gene expansion carriers; M, manifest gene expansion carriers; CSF, cerebrospinal fluid; HP, High performance; MS, mass spectrometry; HPLC, high-performance liquid chromatography; HPressureLC, high pressure liquid chromatography.

Table 6. Summary of published studies on transcriptomic biofluid biomarkers in HD.

Molecule	Sample	Study	n	Direction	Method
Multiple mRNA	Blood	Borovecki 2005 [92]	71	C < HDCorr stage	QRT-PCR
IER3 mRNA	Blood	Runne 2007 [93]	90	C < HD	QRT-PCR
Chromosomal variation	Blood	Anderson 2008 [177]	31	C < HD C < PM	QRT-PCR
“12-gene set”	Blood	Lovrecic 2009 [178]	123	C < HD	QRT-PCR
ARFGEF2 and GOLGA8G	Blood	Lovrecic 2010 [179]	46	C < HD	QRT-PCR
H2AFY	Blood	Hu 2011 [180]	119	C < HD	QRT-PCR
MAOB, TGM2, SLC2A4, BCKDK	Blood	Krzyszton-Russjan 2013 [149]	57	C < HD	QRT-PCR
LDHA, BDNF	Blood	Krzyszton-Russjan 2013 [149]	57	C > HD	QRT-PCR
TLR2, LTBR, CD40, TMED4, AKT1, IL10, FR2	Blood	Trager 2014 [142]	20	C < HD	RT-PCR
CHUK		Trager 2014 [142]	20	C > HD	RT-PCR
SERCA2 and VEGF	Blood	Cesca 2015 [181]	164	C > HD	RT-PCR
miRNA-34b	Blood	Gaughwin 2011 [182]	23	C < PM	miRNA RT-PCR
ND1, COX1, CYTB mtRNA	Blood	Chen 2007 [119]	52	C = HD	QRT-PCR

Abbreviations: n, sample size; C, healthy controls; HD, Huntington’s disease gene expansion carriers; PM, premanifest gene expansion carriers; M, manifest gene expansion carriers;mRNA, RNA of mtDNA-encoded mitochondrial enzymes; mtDNA, mitochondrial DNA;; QRT-PCR , Quantitative Real-time PCR; RT-PCR , Real-time PCR;

Table 7. Summary of published studies on proteomic biofluid biomarkers in HD.

Sample	Study	n	Direction	Method
CSF	Dalrymple 2007 [51]	29	Corr stage	ELISA
	Fang 2008 [183]	30	CHGB, SIAE, IDS, NRXN3, GSN, ENDOD1, GRIA4, GGH, GC, C4B, PRNP: C > HD C1QC, HPX, TPI1, PKM2/PKLR, LYZ, FAM3C, LMAN2: C < HD	5 diff methods (1 per lab
	Huang 2011 [147]	12 18	Prothrombin, ApoA-IV, Haptoglobin: C < HD Prothrombin: C < HD ApoA-IV: C < HD Haptoglobin: C < HD	2D Electrophoresis + MS Western blot analysis or
	Vinther-Jensen 2015 [184]	121	10 peaks (Ubiquitin, transthyretin)	SELDI-TOF MS
Blood	Dalrymple 2007 [51]	20	b-actin, ApoA-IV, clusterin: C < HD	2D Gel Electrophoresis +
		109	C7, C9, a2-macroglobulin, a2-antiplasmin: C < HD Afamin, IGF binding protein, PRBP: C > HD	2D Gel Electrophoresis +
		60	a-/b-clusterin: C < HD, corr Stage	Semiquantitative Immun
		73	Corr stage	ELISA
		96	Corr stage	ELISA
	Huang 2011 [147]	18	Prothrombin: C = HD ApoA-IV: C = HD Haptoglobin: C = HD	Western blot analysis or

Abbreviations: n, sample size; C, healthy controls; HD, Huntington's disease gene expansion carriers; PM, premanifest gene expansion carriers; M, manifest gene expansion carriers; CSF, cerebrospinal fluid; Corr, Correlation; ELISA, enzyme-linked immunosorbent assay; MS, mass spectrometry; SELDI-TOF MS, Surface-enhanced laser desorption/ionization time-of-flight mass spectrometry; LC/MS/MS, Liquid chromatography tandem mass spectrometry.

Table 8. Summary of published studies on kynurenine pathway metabolites in HD.

Molecule	Sample	Study	n	Direction	Method
Quinolinic acid	CSF	Schwarcz 1988 [94]	17	C = HD	Radioenzymatic assay
		Heyes 1991 [95]	18	C = HD	N/S
		Heyes 1992 [96]	43	C = HD	HPLC
	Blood	Stoy 2005 [29]	26	C = HD	GC/MS
	Urine	Heyes 1985 [185]	25	C = HD	HPressureLC
Quinolinic acid phophoribosyltransferase	Blood	Foster 1985 [186]	6	C = HD	Scintillation spectrometry
Kynurenic acid	CSF	Beal 1990 [97]	73	C > HD	HPLC
		Heyes 1992 [96]	43	C > HD	HPLC
	Blood	Stoy 2005 [29]	26	C = HD	HPLC
		Forrest 2010 [102]	113	C = HD	HPLC fluorescence detection
Kynurenine	CSF	Beal 1990 [97]	73	C > HD	HPLC
		Heyes 1992 [96]	43	C > HD	HPLC
	Blood	Leblhuber 1998 [140]	23	C = HD	HPLC
		Stoy 2005 [29]	26	C < HD	HPLC
		Forrest 2010 [102]	113	C = HD	HPLC absorbance detection spectrometry
3-hydroxykynurenine	Blood	Stoy 2005 [29]	26	C > HD	HPLC
Tryptophan	CSF	Heyes 1992 [96]		C = HD	HPLC
		García Ruiz 1995 [85]	35	C = HD	HPressureLC
	Blood	Phillipson 1977 [151]	18	C = HD	[187]
		Belendiuk 1980 [80]	76	C > HD	Fluorometry
		Leblhuber 1998 [140]	23	C > HD	HPLC
		Stoy 2005 [29]	26	C = HD	HPLC
		Christofides 2006 [91]	26	C = HD	HPLC
		Forrest 2010 [102]	113	C = HD	HPLC absorbance detection spectrometry
3-hydroxyanthranilic acid	Blood	Stoy 2005 [29]	26	C > HD	HPLC
		Forrest 2010 [102]	113	C = HD	HPLC fluorescence detection

Anthranilic acid	Blood	Forrest 2010 [102]	113	C = HD	HPLC fluorescence detection
Xanthurenic acid	Blood	Stoy 2005 [29]	26	C > HD	HPLC

Abbreviations: n, sample size; C, healthy controls; HD, Huntington's disease gene expansion carriers; PM, premanifest gene expansion carriers; M, manifest gene expansion carriers; CSF, cerebrospinal fluid; HPLC, high-performance liquid chromatography; GC/MS, gas chromatography mass spectrometry; HPressureLC, high pressure liquid chromatography.

Table 9. Summary of published studies on oxidative stress biofluid biomarkers in HD.

Molecule	Sample	Study	n	Direction	Method
F2-isoprostanes	CSF	Montine 1999 [109]	43	C < HD	[106]
	Blood	Montine 2000 [110]	42	C = HD	Highly accurate quantitative MS
	Urine	Montine 2000 [110]	33	C = HD	Highly accurate quantitative MS
8OH2'dG	Blood	Hersch 2006 [114]	94	C < HD ↓ w/ creatine treatment (HD)	[188]
		Chen 2007 [119]	52	C < HD	HPLC-ECD
		Biglan 2012 [120]	28	C = HD ↓ w/ creatine treatment (HD+C)	[188]
		Long 2012 [121]		C = PM ↑ w/ proximity to onset	Long gradient Liquid Chromatography Electrochemical Array and/or Liquid Chromatography MS
		Borowsky 2013 [122]	160	C = PM No diff longitudinally	Liquid chromatography–electrochemical array (LCECA) assay or liquid chromatography–MS (LCMS).
		Ciancarelli 2014 [189]	28	C < HD No corr	ELISA
		Rosas 2014[115]	64	C = PM = w/ creatine treatment	N/S
		Ciancarelli 2015 [190]	34	C < HD = w/ neurorehabilitation No corr	ELISA
Malondialdehyde	Blood	Stoy 2005 [29]	26	C < HD	Bioxytech LPO-586 colourimetric assay
		Christofides 2006 [91]	26	C < HD	Bioxytech LPO-586 colourimetric assay

		Chen 2007 [119]	52	C < HD Corr TMS and IS	HPLC
		Forrest 2010 [102]	111	C = HD	Bioxytech LPO-586 colorimetric assay
		Pena-Sanchez 2015 [191]	53	C < HD	Bioxytech LPO-586 colourimetric assay
	Urine	Olsson 2012 [192]	Sample1= 130 Sample2= 73	C = HD	Thiobarbituric acid method
4-hydroxynonenal	Blood	Stoy 2005 [29]	26	C < HD	Bioxytech LPO-586 colourimetric assay
		Christofides 2006 [91]	26	C < HD	Bioxytech LPO-586 colourimetric assay
		Forrest 2010 [102]	111	C = HD	Bioxytech LPO-586 colorimetric assay
AOPPs	Blood	Pena-Sanchez 2015 [191]	53	C < HD Corr age of onset Corr TMS Corr age	[193]
Superoxide dismutase	Blood	Chen 2007 [119]	52	C > HD	RANSOD kit
		Klepac 2007 [194]	99	C = HD	Spectrophotometrically
		Ciancarelli 2014 [189]	28	C < HD No corr	ELISA
		Ciancarelli 2015 [190]	34	C < HD ↓ w/ neurorehabilitation No corr	ELISA
		Pena-Sanchez 2015 [191]	53	C = HD	[195]
Catalase	Blood	Klepac 2007 [194]	99	C = HD	Spectrophotometrically

		Pena-Sanchez 2015 [191]	53	C = HD	[196]
glutathione peroxidase	Blood	Chen 2007 [119]	52	C > HD PM = M	[197]
		Pena-Sanchez 2015 [191]	53	C < HD	[197]
glutathione reductase	Blood	Pena-Sanchez 2015 [191]	53	C > HD	[198]
reduced glutathione	Blood	Klepac 2007 [194]	99	C > HD	Spectrophotometrically
		Pena-Sanchez 2015 [191]	53	C = HD Corr caudate vol	Bioxytech GSH/GSSG-412
protein thiols and total antioxidant capacity	Blood	Pena-Sanchez 2015 [191]	53	C = HD Corr age of onset	[199]
a_1-microglobulin	Blood	Olsson 2012 [192]	67 (cohort 2)	C =HD	Radioimmunoassay
	Urine	Olsson 2012 [192]	130 (cohort 1) 73 (cohort 2)	C < HD, C < PM, C< eM C < HD, C < PM	Radioimmunoassay
Protein carbonyl groups	Blood	Olsson 2012 [192]	67 (cohort 2)	C =HD	Absorbance test
	Urine	Olsson 2012 [192]	130 (cohort 1) 73 (cohort 2)	C = HD C = HD	Absorbance test
NSE	Blood	Ciancarelli 2014 [189]	28	C < HD No corr	ELISA
		Ciancarelli 2015 [190]	34	C < HD	ELISA

				↓ w/ neurorehabilitation No corr	
Haemoglobin	Blood	Leblhuber 1998 [140]	23	C = HD	N/S
		Olsson 2012 [192]	67 (cohort 2)	C = HD	ELISA
	Urine	Olsson 2012 [192]	73 (cohort 2)	C < eM C < mM Corr TFC	ELISA
Protein carbonyl groups	Urine	Olsson 2012 [192]	73	C = HD	ELISA
Malonyldialde hyde	Blood	Forrest 2010 [102]	113	C = HD	Bioxytech LPO-586 colorimetric assay
		Olsson 2012 [192]	67 (cohort 2)	C = HD	Thiobarbituric acid method
	Urine	Olsson 2012 [192]	73 (cohort 2)	C = HD	Thiobarbituric acid method
Nitric oxide	Blood	Sanchez-Lopez 2012 [141]	23	C = HD	Gries method
		Carrizzo 2014 [200]	83	C > HD eHD > aHD	HPLC
Nitric oxide synthase	Blood	Sanchez-Lopez 2012 [141]	23	C = HD	Ultrasensitive Colorimetric NOS Assay
		Carrizzo 2014 [200]	83	C > HD eHD > aHD	[201]
Nitrotyrosine	Blood	Tasset 2012 [128]	38	C < HD	N/S
Thioredoxin reductase-1	Blood	Sanchez-Lopez 2012 [141]	23	C > HD	ELISA
Thioredoxin-1	Blood	Sanchez-Lopez 2012 [141]	23	C > HD	ELISA
Lipid peroxide	Blood	Duran 2010 [152]	91	C < M	PeroxiDetect (Sigma) Enzymatic Bioanalysis (Roche) kit

Aminopeptidases	Blood	Duran 2010 [152]	91	C > HD PM = M	[202]
Aspartate	Blood	Reilmann 1994 [203]	37	C > HD	HPLC
Glutamate	CSF	Kim 1980 [204]	16	C > HD	[205]
		Kim 1980 [204]	16	C =HD	[205]
	Blood	Reilmann 1994 [203]	37	C = HD	HPLC
Glutamine	Blood	Reilmann 1994 [203]	37	C = HD	HPLC
L-pyroglutamin acid	CSF	Uhlhaas 1988 [206]	50	C = HD	N/S
	Blood	Uhlhaas 1988 [206]	50	C < HD Corr disease duration	N/S
Phenylalanine	Blood	Reilmann 1994 [203]	37	C = HD	HPLC

Abbreviations: n, sample size; C, healthy controls; HD, Huntington's disease gene expansion carriers; PM, premanifest gene expansion carriers; M, manifest gene expansion carriers; eHD, early HD; aHD, advanced HD; TMS, UHDRS Total Motor Score; CSF, cerebrospinal fluid; MS, mass spectrometry; HPLC-ECD, high performance liquid chromatography-electrochemical detector; ELISA, enzyme-linked immunosorbent assay; HPLC, high-performance liquid chromatography.

Table 10. Summary of published studies on neuroendocrine biofluid biomarkers in HD.

Molecule	**Sample**	**Study**	**n**	**Direction**	**Method**
Growth hormone	CSF	Caraceni 1977[89]	14	C = HD	Radioimmunoassay
	Blood	Keogh 1976 [207]	12	C = HD	Radioimmunoassay
		Phillipson 1976 [208]	18	C < HD	[209]
		Chalmers 1978 [210]	19	C = HD	Radioimmunoassay
		Muller 1979 [211]	45	C = HD	N/S
		Murri 1980 [212]	12	C = HD	Radioimmunoassay
		Lavin 1981 [213]	18	C = HD	Radioimmunoassay
		Durso 1983 [214]	18	C < HD	Radioimmunoassay
		Durso 1983 [215]	16	C = HD	Radioimmunoassay
		Popovic 2004 [216]	35	C = HD	Time-resolved fluoro-immunometric assay
		Saleh 2009 [217]	290	C < HD C < HD1/2 Asso IS, TA, TFC, TMS	ELECSYS2010 kit
		Aziz 2010 [218]	18	C = HD	Time-resolved fluoroimmunoassay
		Saleh 2010 [219]	109	Asso Cogn (males)	ACCESS2 kit
		Salvatore 2011 [220]	34	C < HD	Radioimmunoassay
		Wang 2014 [132]	39	C > HD	Human Brain-Derived/Pituitary Protein Multiplex Panel assay kit
IGF1	Blood	Popovic 2004 [216]	35	C = HD	Enzyme-labeled chemiluminescent immunometric assay
		Mochel 2007 [139]	53	C > HD Corr TMS	PNMRS

		Saleh 2009 [217]	290	C < HD Asso IS, FA, TFC	IMMULITE2500 kit
		Aziz 2010 [218]	18	C = HD	Radioimmunoassay
		Saleh 2010 [219]	109	Asso Cogn (males)	IMMULITE2500kit
		Salvatore 2011 [220]	34	C > HD	Radioimmunoassay
		Russo 2013 [221]	56	C > HD	Radioimmunoassay
IGF binding protein	Blood	Dalpymple 2007	109	C > HD	2D electrophoresis
		Aziz 2010 [218]	18	C = HD	Radioimmunoassay
Prolactin	Blood	Caraceni 1977[89]	14	C < HD	Radioimmunoassay
		Hayden 1977 [222]	20	C > HD	Radioimmunoassay
		Caine 1978 [223]	15	C < HD	Radioimmunoassay
		Chalmers 1978 [210]	19	C = HD	Radioimmunoassay
		Murri 1980 [212]	12	C = HD	Radioimmunoassay
		Lavin 1981 [213]	18	C = HD	Radioimmunoassay
		Durso 1983 [214]	18	C = HD	Radioimmunoassay
		Durso 1983 [215]	16	C = HD	Radioimmunoassay
		Kremer 1989 [224]	20	C = HD = after 2y	Radioimmunoassay
		Saleh 2009 [217]	290	C = HD	ACCESS2 kit
		Markianos 2009 [87]	172	C = PM C < M	Radioimmunoassay
		Aziz 2010 [225]	18	C = HD	Time-resolved immunofluorometic assays
		Wang 2014 [132]	39	C > HD	Human Brain-Derived/Pituitary Protein Multiplex Panel assay kit
CRF	CSF	Kurlan 1988 [90]	77	C < HD Corr	Radioimmunoassay

				depression	
ACTH	Blood	Heuser 1991 [226]	20	C < HD	[227]
		Saleh 2009 [217]	290	C = HD	ELECSYS2010 kit
		Russo 2013 [221]	56	C > HD	Immunoassay
Cortisol	Blood	Bruyn 1972 [228]	12	Low in 8 patients	N/S
		Lavin 1981 [213]	18	C = HD	Radioimmunoassay
		Heuser 1991 [226]	20	C < HD	[227]
		Leblhuber 1995 [229]	36	C < HD	DHEAS -Diagnostic Products Corporation, CRT Abbot
		Markianos 2007 [230]	125	C = PM = M	N/S
		Mochel 2007 [139]	53	C = HD	8089-K kit
		Saleh 2009 [217]	290	C < HD	ELECSYS2010 kit
		Krzyszton-Russjan 2013	57	C = HD	N/S
		Aziz 2009 [231]	16	C < HD Corr TMS, TFC, BMI	Radioimmunoassay
		Russo 2013 [221]	56	C = HD	Immunoassay
	Urine	Bjorkqvist 2006	150	C < HDIII and IV C = PM and HDI and II	Radioimmunoassay
LH	Blood	Lavin 1981 [213]	18	C = HD	Radioimmunoassay
		Markianos 2005 [232]	86	C > HD	Radioimmunoassay
		Saleh 2009 [217]	146	C = HD	ELECSYS2010 kit
FSH	Blood	Lavin 1981 [213]	18	C = HD	Radioimmunoassay
		Markianos 2005 [232]	86	C > HD	Radioimmunoassay
		Saleh 2009 [217]	146	C = HD	ELECSYS2010 kit

Testosterone	Blood	Bruyn 1972 [228]	12	HD within normal limits	Not stated
		Markianos 2005 [232]	86	C > HD	Radioimmunoassay
		Markianos 2007 [230]	125	C = PM = M	N/S
		Saleh 2009 [217]	146	C = HD Asso IS, FA, TFC, Behaviour	ELECSYS2010 kit
		Russo 2013 [221]	19	C = HD	Immunoassay
DHEA	Blood	Bruyn 1972 [228]	12	HD within normal limits	Not stated
DHEA-sulphate	Blood	Bruyn 1972 [228]	12	Low in 9 patients	Not stated
		Leblhuber 1995 [229]	36	C > HD	DHEAS -Diagnostic Products Corporation, CRT Abbot
		Markianos 2007 [230]	125	C = PM = M	N/S
17-β-estradiol	Blood	Russo 2013 [221]	9	C > HD	Immunoassay
TSH	Blood	Lavin 1981 [213]	18	C = HD	Radioimmunoassay
		Leblhuber 1998[140]	23	C = HD	N/S
		Mochel 2007 [139]	53	C = HD	8089-K kit
		Saleh 2009 [217]	290	C = HD Asso IS, FA, TFC	ELECSYS2010 kit
		Aziz 2010 [225]	18	C = HD	Time-resolved immunofluorometic assays
		Russo 2013 [221]	56	C = HD	Immunoassay
T3	Blood	Lavin 1981 [213]	18	C = HD	Serum total thyroxine minus uptake of ^{125}I labelled triiodothyronine
		Leblhuber 1998[140]	23	C = HD	N/S
		Saleh 2009 [217]	290	C = HD	ELECSYS2010 kit

				Asso IS, FA, TFC, TMS	
		Russo 2013 [221]	56	C = HD	Immunoassay
T4	Blood	Leblhuber 1998 [140]	23	C = HD	N/S
		Mochel 2007 [139]	53	C = HD	8089-K kit
		Saleh 2009 [217]	290	C = HD	ELECSYS2010 kit
Ghrelin	CSF	Popovic 2004 [216]	35	C = HD	Radioimmunoassay
	Blood	Popovic 2004 [216]	35	C < HD	Radioimmunoassay
		Mochel 2007 [139]	53	C < HD (male) C = HD (female)	Radioimmunoassay
		Aziz 2010 [218]	18	C = HD	Radioimmunoassay
		Wang 2014 [132]	39	C < PM	ELISA
Leptin	CSF	Popovic 2004 [216]	35	C = HD	Radioimmunoassay
	Blood	Popovic 2004 [216]	35	C > HD	Radioimmunoassay
		Mochel 2007 [139]	53	C > HD (male) C = HD (female)	Radioimmunoassay
		Aziz 2010 [233]	18	C = HD	Radioimmunoassay
		Wang 2014 [132]	39	C = HD	human gut hormone multiplex kit
Orexin A/Hypocretin-1	CSF	Gaus 2005 [234]	20	C = HD	Radioimmunoassay
		Meier 2005 [69]	22	C = HD	Radioimmunoassay
		Bjorkqvist 2006 [235]	67	C = HD	Radioimmunoassay
Adiponectin	Blood	Aziz 2010 [233]	18	C = HD	Radioimmunoassay
		Wang 2014 [132]	39	C = HD	Radioimmunoassay
Resistin	Blood	Aziz 2010 [233]	18	C = HD	Radioimmunoassay
CART	CSF	Bjorkqvist 2007	67	C < HD	Radioimmunoassay

		[236]			
BDNF	Blood	Ciammola 2007 [127]	84	C > HD Corr TFC Corr Cogn	ELISA
		Squitieri 2009 [129]	142	C > PM	ELISA
		Squitieri 2009 [144]	N/S	C > HD	ELISA
		Zuccato 2011 [130]	398	C = HD	ELISA
		Tasset 2012 [128]	38	C > HD	ELISA
		Wang 2014 [132]	39	C = HD	Human Brain-Derived/Pituitary Protein Multiplex Panel assay kit
NGF	Blood	Lorigados 1992 [237]	9	C > HD	ELISA
		Tasset 2012 [128]	38	C > HD eHD > aHD	ELISA
GDNF	Blood	Squitieri 2009 [144]	N/S	C = HD	ELISA
		Tasset 2012 [128]	38	C > HD	ELISA
Anandamide	Blood	Battista 2007 [238]	14	C < HD	HPLC
Vasopressin/ADH	Blood	Wood 2008 [239]	107	C < HD	Radioimmunoassay
Osmolality	Blood	Wood 2008 [239]	107	C = HD	Not stated
Insulin	Blood	Keogh 1976 [207]	12	C = HD	Immunoreactive insulin
		Kremer 1989 [224]	20	C = HD = after 2y	Radioimmunoassay
		Popovic 2004 [216]	35	C = HD	Radioimmunoassay
		Lalic 2008 [240]	51	C < HD	Radioimmunoassay
		Salvatore 2011 [220]	34	C = HD	Not stated

		Russo 2013 [221]	56	C = HD	Not stated
		Wang 2014 [132]	39	C = HD	human gut hormone multiplex kit
Glucose	Blood	Keogh 1976 [207]	12	C = HD	Glucose oxidase method
		Podolsky 1977 [241]	14	Normal values	Glucose oxidase method
		Caine 1978 [223]	15	C = HD	Not stated
		Phillipson 1977 [151]	18	C = HD	Standard neocuproine method
		Lavin 1981 [213]	18	C = HD	Glucose oxidase method
		Kremer 1989 [224]	20	C = HD = after 2y	Standard hexokinase method
		Leblhuber 1998 [140]	23	C = HD	N/S
		Popovic 2004 [216]	35	C = HD	Beckman Glucose Analyzer 2
		Lalic 2008 [240]	51	C = HD	Glucose oxidase method
		Josefsen 2010 [153]	19	C = HD	[242]
		Salvatore 2011 [220]	34	C = HD	Not stated
		Krzyszton-Russjan 2013 [149]	57	C = HD	N/S
		Russo 2013 [221]	56	C = HD	Not stated
		Wang 2014 [132]	39		Glucose assay kit
C-peptide	Blood	Kremer 1989 [224]	20	C = HD = after 2y	Radioimmunoassay
HbA1c	Blood	Kremer 1989 [224]	20	C = HD = after 2y	HPLC
Amylin	Blood	Wang 2014 [132]	39	C < HD	Human gut hormone multiplex kit
Glucagon	Blood	Wang 2014 [132]	39	C > M	Millipore RIA kit
Somatomedin-C	Blood	Kremer 1989 [224]	20	C = HD	Radioimmunoassay

				= after 2y	
GIP	Blood	Wang 2014 [132]	39	C = HD	Human gut hormone multiplex kit
PYY	Blood	Wang 2014 [132]	39	C = HD	Human gut hormone multiplex kit
PP	Blood	Wang 2014 [132]	39	C = HD	Human gut hormone multiplex kit
Melatonin	Blood	Christofides 2006 [91]	26	C = HD	Direct radioimmunoassay [243]
		Aziz 2009 [134]	18	C = HD	Radioimmunoassay
		Kalliolia 2014 [135]	42	C = PM C > M	Radioimmunoassay
Plasma retinol binding protein	Blood	Dalrymple 2007 [51]	109	C > HD	2D electrophoresis
Osteocalcin	Blood	Silajdzic 2013 [61]	Cohort1 79	C = HD	MSD Immunoassay
Osteonectin	Blood	Silajdzic 2013 [61]	Cohort1 79	C = HD	MSD Immunoassay
Osteopontine	Blood	Silajdzic 2013 [61]	Cohort1 79	C = HD	MSD Immunoassay
PEGF	Blood	Silajdzic 2013 [61]	Cohort1 79	C = HD	Luminex

Abbreviations: n, sample size; C, healthy controls; HD, Huntington's disease gene expansion carriers; PM, premanifest gene expansion carriers; M, manifest gene expansion carriers; eHD, early HD; aHD, advanced HD; Corr, correlation; TFC, UHDRS Total Functional Capacity; Cogn, measures of cognition; PNMRS, proton nuclear magnetic resonance spectroscopy; MSD, Meso ScaleDiscovery; HPLC, high-performance liquid chromatography;

Table 11. Summary of published studies on metabolic biofluid biomarkers in HD.

Molecule	Sample	Study	n	Direction	Method
ApoA-I	Blood	Silajdzic 2013 [61]	Cohort1 79	C = HD	Luminex
ApoA-IV	CSF	Huang 2011 [147]	12	C < HD	Two-dimensional electrophoresis and mass spectrometry
		Huang 2011 [147]	18	C < HD	ELISA
	Blood	Huang 2011 [147]	18	C = HD	ELISA
	Blood	Dalrymple 2007 [51]	20	C < HD	2D electrophoresis
ApoC3	Blood	Silajdzic 2013 [61]	Cohort1 79	C = HD	Luminex
ApoE	Blood	Silajdzic 2013 [61]	Cohort1 79 Cohort2 42	C = HD C = HD	Luminex
BCAA	Blood	Phillipson 1977 [151]	18	C > HD	Durrum D500 Automatic Amino Acid Analyser
		Mochel 2007 [139]	53	C > HD	PNMRS
Valine	Blood	Phillipson 1977 [151]	18	C > HD	Durrum D500 Automatic Amino Acid Analyser
		Mochel 2007 [139]	53	C > HD	Ion exchange chromatography
Leucine	Blood	Phillipson 1977 [151]	18	C > HD	Durrum D500 Automatic Amino Acid Analyser
		Mochel 2007 [139]	53	C > HD Corr TFC	Ion exchange chromatography
Isoleucine	Blood	Phillipson 1977 [151]	18	C > HD	Durrum D500 Automatic Amino Acid Analyser
		Mochel 2007 [139]	53	C > HD Corr TFC	Ion exchange chromatography
Cystathionine	Blood	Aziz 2015 [244]	18	C = HD	[245]
	Urine	Aziz 2015 [244]	18	C = HD	[245]
Copper	Blood	Forrest 1957 [246]	12	C = HD	Not stated

Magnesium	Blood	Bruyn 1965 [247]	55	C = HD	Direct spectrophotometric estimation
Iron	Blood	Bonilla 1991 [248]	104	C = HD	Flameless atomic absorption spectrophotometry
		Morrison 1994 [249]	190	C = HD	Not stated
Ferritin	Blood	Bonilla 1991 [248]	104	C > HD	Radioimmunoassay
		Morrison 1994 [249]	190	C > HD	Not stated
TIBC	Blood	Morrison 1994 [249]	190	C = HD	Not stated
Vitamin B12	Blood	Bonilla 1991 [248]	104	C = HD	Radioassay
		Leblhuber 1998 [140]	23	C = HD	Not stated
Folic Acid	Blood	Bonilla 1991 [248]	104	C = HD	Radioassay
		Leblhuber 1998 [140]	23	C = HD	Not stated
Total cholesterol	Blood	Leoni 2008 [250]	191	C = HD	Standard spectophotometry
		Lalic 2008 [240]	51	C = HD	Not stated
		Markianos 2008 [251]	295	C > M C > PM	Commercially available kit ELITech
		Leoni 2011 [252]	303	eHD > aHD	Isotope dilution mass spectrometry
		Salvatore 2011 [220]	34	C = HD	Not stated
		Russo 2013 [221]	56	C = HD	Not stated
		Wang 2014 [132]	39	C > HD	Enzymatic assay kits
		Ciancarelli 2015 [190]	28	C = HD	Architect c8000
Lanosterol	Blood	Leoni 2011 [252]	303	C > M	Isotope dilution mass spectrometry
Lathosterol	Blood	Leoni 2011 [252]	303	C > PM C > M	Isotope dilution mass spectrometry
HDL	Blood	Lalic 2008 [240]	51	C = HD	Not stated

		Salvatore 2011 [220]	34	C = HD	Not stated
		Russo 2013 [221]	56	C > HD	Not stated
		Wang 2014 [132]	39	C = HD	Enzymatic assay kits
		Ciancarelli 2015 [190]	28	C = HD	Architect c8000
LDL	Blood	Lalic 2008 [240]	51	C = HD	Not stated
		Salvatore 2011 [220]	34	C = HD	Not stated
		Russo 2013 [221]	56	C = HD	Not stated
		Krzyszton-Russjan 2013	57	C = HD	N/S
		Wang 2014 [132]	39	C > HD	Enzymatic assay kits
FFA/triglycerides	Blood	Phillipson 1977 [151]	18	C < HD	[253]
		Lalic 2008 [240]	51	C = HD	Not stated
		Russo 2013 [221]	56	C = HD	Not stated
		Wang 2014 [132]	39	C = HD	Enzymatic assay kits
FAAH	Blood	Battista 2007 [238]	113	C > M C > PM	ELISA
24S-hydroxycholesterol	Blood	Leoni 2011 [252]	191	C > PM C > M	Gas chromatography-mass spectrometry
27-hydroxycholesterol	Blood	Leoni 2011 [252]	191	C > PM C > M	Gas chromatography-mass spectrometry
CK-BB	Blood	Kim 2010 [254]	50	C > M C > PM	Dot blot analysis (Western blot?)
Afamin	Blood	Dalrymple 2007 [51]	109	C > HD	2D electrophoresis

Abbreviations: n, sample size; C, healthy controls; HD, Huntington's disease gene expansion carriers; PM, premanifest gene expansion carriers; M, manifest gene expansion carriers; eHD, early HD; aHD, advanced HD; Corr, correlation; TFC, UHDRS Total Functional Capacity; ELISA, ELISA, enzyme-linked immunosorbent assay; PNMRS, proton nuclear magnetic resonance spectroscopy.